PLANT PHYSIOLOGY

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ERRATA

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Page 23, author's name, for "G. PRESTON" read C. PRESTON.
Page 26, table II, delete "gm." in column heading above 5.57.
Page 27, table III, heading, last column, for "per disc" read per 10 discs.
Page 53, line 5 from bottom, for "Some (19)" read Some (19) see.
Page 341, citation no. 6, for 6-em dash read JONES, L. R.
Page 349, line 10, for "tomato, sunflower," read tomato, and sunflower.
Page 349, line 11, delete "and pea."
Page 353, citation 9, for "BARTLETT," read BARTLEY.
Page 474, standard error formula, transpose numerator and denominator.

Vol. 15 No. 1

PLANT PHYSIOLOGY

JANUARY, 1940

RESPONSES OF THE BEAN PLANT TO CALCIUM DEFICIENCY

HUGH GAUCH

(WITH SEVENTEEN FIGURES)

Introduction

Calcium has long been regarded as an essential element for most plants, and many rôles have been assigned to it by numerous investigators. It is, however, beyond the scope of this paper to review all of the literature dealing with calcium, or with each of the many rôles that have been attributed to it. The recent review by MILLER (11) is an excellent source for a general survey of the literature.

Many studies have been made comparing the growth of various plants with and without calcium in the nutrient solution. In many cases, however, the solutions have been chosen arbitrarily without a thorough understanding of the effects produced by various combinations of salts. Experiments with various single salts, two-salt combinations, and various total concentrations of salts were deemed necessary in order to obtain the most favorable plus- and minus-calcium solutions for the growth of the bean plant. The differences between the plants growing in these two solutions are believed to indicate more truly the morphological and chemical alterations that result from a deficiency of calcium in the nutrient solution.

Materials and methods

The plant used in this study was the Dwarf Red Kidney bean, *Phaseolus vulgaris*. The seeds were obtained from a commercial grower of beans at Marysville, California. In these studies the seeds were planted at a depth of one inch in typical greenhouse flats containing unused white, coarse sand, and germinated in darkness for seven days. The sand was kept moist by additions of tap water (containing approximately 32, 11, 1, 0.1, and 15 p.p.m. of calcium, magnesium, potassium, nitrate, and sulphate, respectively). Bean seeds are very sensitive during germination to an over- or an under-supply of

moisture. Temperature in the dark room fluctuated from 78° to 80° F., and the relative humidity varied from 30 to 50 per cent. The seedlings were from seven to eight days old at the time of transfer to the nutrient solutions (fig. 1).

FIG. 1. Seven-day-old bean seedlings as transferred to nutrient solution.

The flats were flooded with tap water to facilitate removal of the seedlings, and after the sand was washed from the roots, the roots were temporarily immersed in distilled water before planting. In each planting, 360 seedlings were accommodated by the 72 one-half gallon glazed crocks, *i.e.*, five plants per crock. The crocks were covered with sheets of cork (paraffined), $6'' \times 6'' \times \frac{1}{4}''$, which served as supports for the plants. Nonabsorbent cotton was pressed lightly into the space between the hypocotyl and the cork support (fig. 4). Early experiments on bean showed that aeration of the cultures was unnecessary for the length of the growth period under study.

As mentioned earlier, many single salts as well as two-salt combinations in various total concentrations were tested; the details of these experiments are reported later. The growth responses—morphological and chemical—are reported in detail for two types of plus- and minus-calcium solutions: (1) those used by NIGHTINGALE, *et al.* (12) (designated "solutions A+ and A–"),

TABLE I

COMPOSITION OF THE PLUS- AND MINUS-CALCIUM SOLUTIONS

SOLUTION AND SYMBOL*	PARTIAL VOLUME-MOLECULAR CONCENTRATION				
	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	KNO_3	$NaNO_3$
Nightingale, *et al.*:					
Plus-calcium (A+)	0.018	0.009	0.009	.....	.....
Minus-calcium (A–)	.....	0.009	0.009	0.026	.....
For bean:					
Plus-calcium (B+)	0.0075	0.005	0.0005	.....	.....
Minus-calcium (B–)	.....	0.005	0.0005	.....	0.015

* Plus 0.5 p.p.m. of boron, as boric acid, and 10 p.p.m. of iron, as ferric citrate.

and (2) solutions adapted to the bean plant (designated "solutions B+ and B–"). Solutions B+ and B– were prepared on the basis of the results obtained from single-salt and two-salt studies covering a wide range of concentrations (table I).

At the time of harvest, the plants were divided into five fractions, *viz.*, root, hypocotyl, cotyledons, primary leaves, and a residual fraction (first internode plus that portion above the primary leaves). The latter fraction is referred to as "first internode and 'top'."

Physical measurements

Fresh weights of plants, or plant fractions, were determined on a torsion balance sensitive to 0.1 gram. Oven-dry weights were determined on plant material that had been dried at 100° C. in an electric oven for 24 hours, after which it was dried to constant weight in a vacuum oven at 80° C. Oven-dry weights were obtained by use of an analytical balance.

Chemical methods

Total nitrogen.—Total nitrogen was determined by the Gunning method modified to include the nitrogen of nitrates (**7**).

Ash determinations.—Duplicate samples of the oven-dry material were ashed to constant weight at a dull-red heat in a muffle furnace.

Calcium determinations.—Calcium was determined, with slight modification, according to the method outlined by Lindner and Kirk (**6**). After ashing, the sample was dissolved in 1:1 hydrochloric acid, and 10 ml. of ammonium oxalate (saturated) plus two drops of brom-cresol-purple, were added. The mixture was heated just to boiling, and dilute ammonium hydroxide was added until the indicator turned purple. After standing overnight for precipitation, the mixture was filtered and the precipitate of calcium oxalate was washed with dilute (10 per cent.) ammonium hydroxide, saturated with calcium oxalate. A special type of sintered-glass filter was used in order that the final titration of the oxalic acid might be carried out in the same Erlenmeyer flask in which the calcium oxalate was precipitated, and hence minimized any loss. The calcium oxalate was dissolved in 2 N sulphuric acid, heated on a hot plate, and 20 ml. of 0.01 N (or 0.1 N, with the larger amounts of calcium) ceric sulphate were added before setting aside to cool. The excess ceric sulphate, that remained after reacting with oxalic acid, was titrated with 0.01 N ferrous ammonium sulphate, using the ortho-phenanthroline-ferrous complex as the indicator.

Experimentation

Single-salt studies

In order that the effect of a single salt alone might be determined, plants were grown in various single-salt solutions over a wide range of concentra-

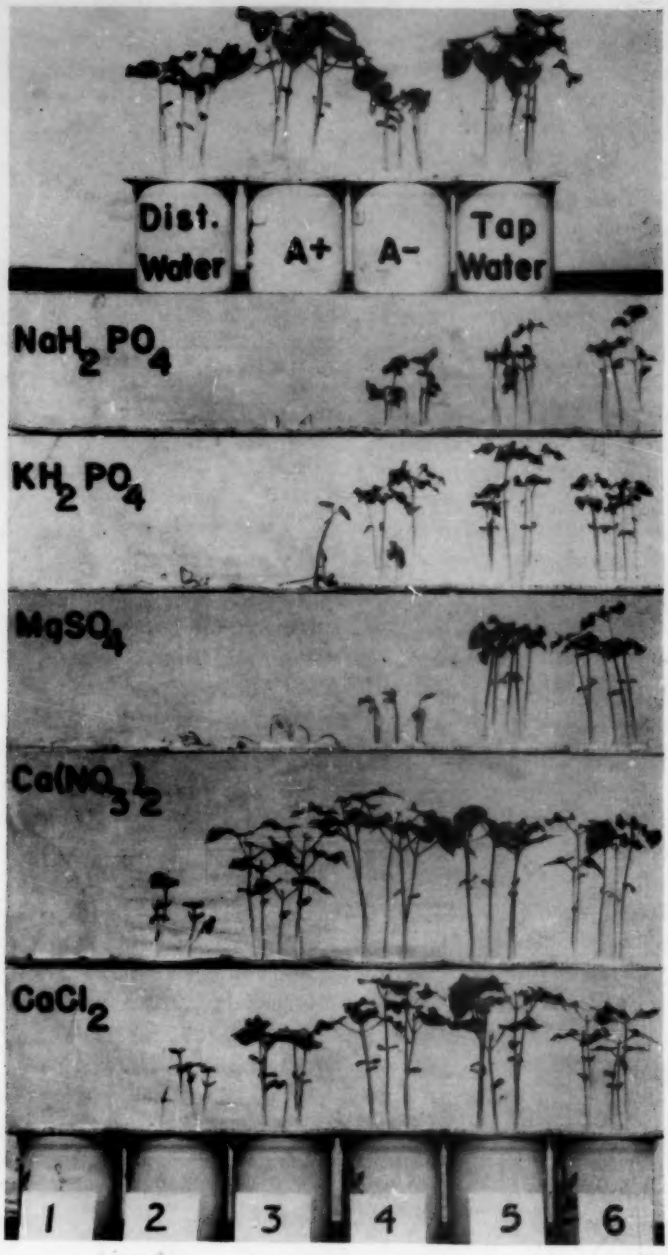

FIG. 2. Bean plants, 9 days after planting into various concentrations of single-salt solutions. Concentrations, vertical rows 1 to 6, are: 0.5, 0.1, 0.05, 0.01, and 0.001 M.

tions, including 0.5, 0.1, 0.05, 0.01, 0.001, and 0.0001 molar. $Ca(NO_3)_2$, $CaCl_2$, $MgSO_4$, $MgCl_2$, KNO_3, KCl, KH_2PO_4, $NaNO_3$, NaCl, NaH_2PO_4, and Na_2SO_4 were used. For all except the two calcium salts, 0.1 and 0.5 M proved to be too concentrated, while the response of plants in the 0.0001 M concentration was essentially like that produced by distilled water (fig. 2). With the exception of Na_2SO_4, which was very toxic, responses in the sodium and potassium salts were much alike. Representative salts of these two cations are shown in figure 2.

High amounts of potassium and sodium produce similar, characteristic symptoms. With an excess of either of these two cations, the hypocotyl becomes more or less water-soaked in appearance and assumes a light tan color.

Little or no root growth resulted in any solutions except those of the calcium salts. With the magnesium salts there was a typical burning of the pulvinus at the base of the primary leaves, and spotting and necrosis of the leaf blades. Moreover, by the second day after planting, the hypocotyls were a very deep, reddish-brown color in the 0.5, 0.1, and 0.05 M concentrations. The coloration first appeared opposite the protoxylem points, and then soon spread throughout the hypocotyl tissue in general.

Two-salt combinations

After determining the effects produced by a number of single salts, two-salt combinations were studied. The three combinations that were studied dealt with $Ca(NO_3)_2$ and $MgSO_4$, $Ca(NO_3)_2$ and KH_2PO_4, and KH_2PO_4 and $MgSO_4$. Each salt in varying amounts (0.05, 0.5, 2.5, 5, 12.5, and 25 ml. of molar salt per liter of solution) was combined separately with each of the other two salts. This method of combining these salts yields 108 different solutions. Dry weight of the tops, minus cotyledons, was taken as the criterion of growth. If one considers the average growth produced by the various amounts of $MgSO_4$ or KH_2PO_4, when plotted against $Ca(NO_3)_2$, on the abscissa, 5 to 12.5 ml. of molar $Ca(NO_3)_2$ per liter of solution is the optimal amount (fig. 3A). Combining $MgSO_4$ with KH_2PO_4 (fig. 3B), 5 ml. molar KH_2PO_4 per liter is optimal; 2.5 to 5 ml. is the optimal amount of molar KH_2PO_4 when combined with $Ca(NO_3)_2$ (fig. 3B). When KH_2PO_4 is combined with $MgSO_4$ (fig. 3C), 0.05 ml. of molar $MgSO_4$ per liter is suboptimal, 0.5 ml. is optimal, and greater amounts of $MgSO_4$ result in progressively less growth of the plants. Also of interest is the fact that with the optimal amount, or less, of $MgSO_4$ there is no magnesium-burning or toxicity. When $Ca(NO_2)_2$ is combined with $MgSO_4$, 0.05 ml. of molar $MgSO_4$ per liter is also limiting, while 0.5 ml. gives optimal and maximal dry weight of plants. Thus it is shown that when $MgSO_4$ is combined with a calcium salt the higher amounts (above optimum) of $MgSO_4$ can be tolerated by the plants with no decrease in the amount of growth. This is not true for the combination of KH_2PO_4 with $MgSO_4$ (fig. 3C).

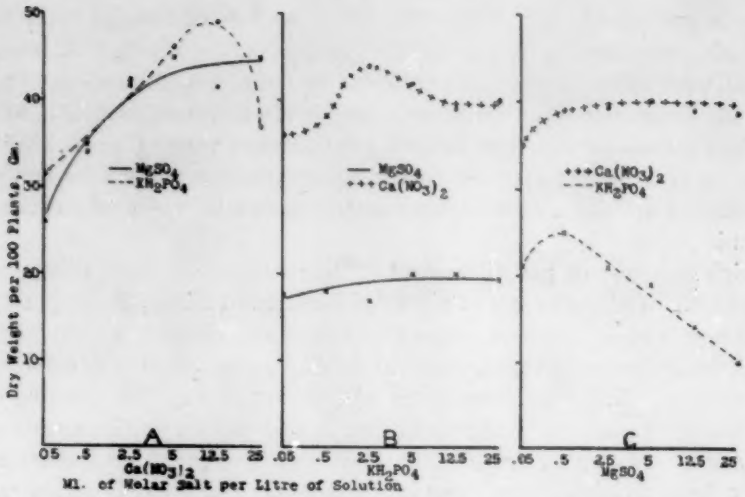

FIG. 3. Growth of bean plants in two-salt solutions. Each curve represents the average growth produced by the various concentrations of a given salt when combined with the salt shown on the abscissa.

On the basis of the results from the single-salt solutions, the two-salt combinations, and the dilution studies (in the following section), the B+ and B– solutions were compounded.

DILUTION STUDIES

The A+ and A– solutions, *i.e.*, 2 atmospheres, and various dilutions of

TABLE II

SIX-DAYS' GROWTH OF BEAN PLANTS IN VARIOUS CONCENTRATIONS OF THE A+ AND A– SOLUTIONS

CONCENTRATION OF SOLUTION	OVEN-DRY WEIGHT PER 100 PLANTS	
	TOPS*	ROOTS
atm.	*gm.*	*gm.*
A+:		
2.0	35.5	4.4
1.0	42.0	5.7
0.1	38.4	5.5
0.01	30.6	5.4
0.001	24.6	3.4
Tap water	30.0	5.3
A–:		
2.0	18.0	0.9
1.0	19.3	1.3
0.1	28.0	2.9
0.01	26.4	3.8
0.001	24.0	3.3
Distilled water	26.6	3.0

* Minus cotyledons.

them (to 1, 0.1, 0.01, and 0.001 atm.) were used, with tap water and distilled water as controls. Dry weights of the tops and roots were taken as the criteria of growth. The results are shown in table II and figure 4.

It is of interest to note that in the A– series the greatest dry weight of tops occurred in the 0.1-atm. concentration. In this concentration the magnesium content is 1/20 of the amount present in the regular A– solution. With a concentration of 0.1 atm. the leaves of the plants did not show magnesium

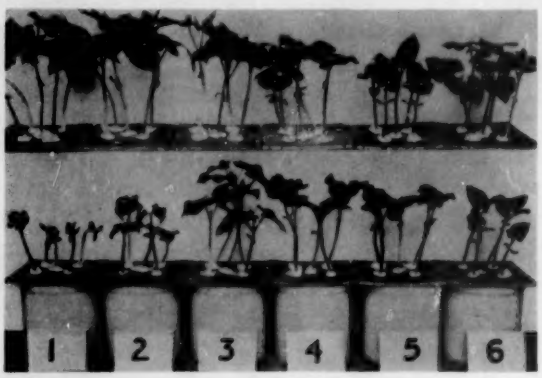

FIG. 4. Bean plants, 5 days after planting, in various dilutions of the A+ and A– solutions. Concentrations are: 2, 1, 0.1, 0.01, and 0.001 atm., crocks 1 to 5; crock 6 is tap water (above), and distilled water (below).

toxicity. This was evidence additional to that obtained in the single- and two-salt studies, suggesting that the magnesium content of a minus-calcium solution should be approximately 0.5 ml. of molar $MgSO_4$ per liter of solution. This amount of $MgSO_4$ was used, therefore, in the B+ and B– solutions as applied to bean plants during the stage of development under study. Other stages of development might very likely require different amounts of magnesium and other ions, as illustrated for wheat by MCCALL and RICHARDS (8).

INTERRELATION OF CALCIUM AND MAGNESIUM

Inasmuch as it is impossible to designate a single value for the Ca/Mg ratio that will prove "best" in all combinations or all concentrations of salts, it seems preferable to speak of the interrelation of calcium and magnesium, potassium, or other ions. The word "ratio" is convenient, however, for the purpose of expressing the relative amounts of one ion to another, and it will be used in that sense only.

In the present study of the interrelation of calcium and magnesium, KH_2PO_4 and KNO_3 were kept constant at 9 and 26 ml. of molar salt per liter, respectively, while $Ca(NO_3)_2$ and $MgSO_4$ were varied to produce Mg/Ca

ratios of 1, 10, 20, 30, 40, and 50. In one series there was 0.18 ml. of molar $Ca(NO_3)_2$ per liter of solution, and the $MgSO_4$ was varied to produce the above-stated ratios. In another series $Ca(NO_3)_2$ was at 1.8 ml.; in still another, at 18 ml. of molar salt per liter of solution, the $MgSO_4$ being varied accordingly. Thus, although the same set of ratios existed in each of the three series, the total concentration of the series varied, as did also the concentration of the members within a series.

A given ratio of Mg/Ca is not the only factor that will determine plant response, as is illustrated in figure 5. Most of the plants in row C were killed by the high concentrations of salts. In row B the various heights of plants in the different crocks are apparently related to the various concentrations. There was little or no magnesium injury in rows A and B with Mg/Ca ratios of 10 or less. This was more obvious in the case of row B than in A, showing that the effect of ratios may be altered by the total concentration of salts. According to OSTERHOUT (13), growth in concentrated (0.1 M) solutions furnishes a much better criterion of antagonism than growth in dilute solutions; at great dilution about the same amount of growth is obtained regardless of the proportions of the two salts. For wheat, TOTTINGHAM (17) and SHIVE

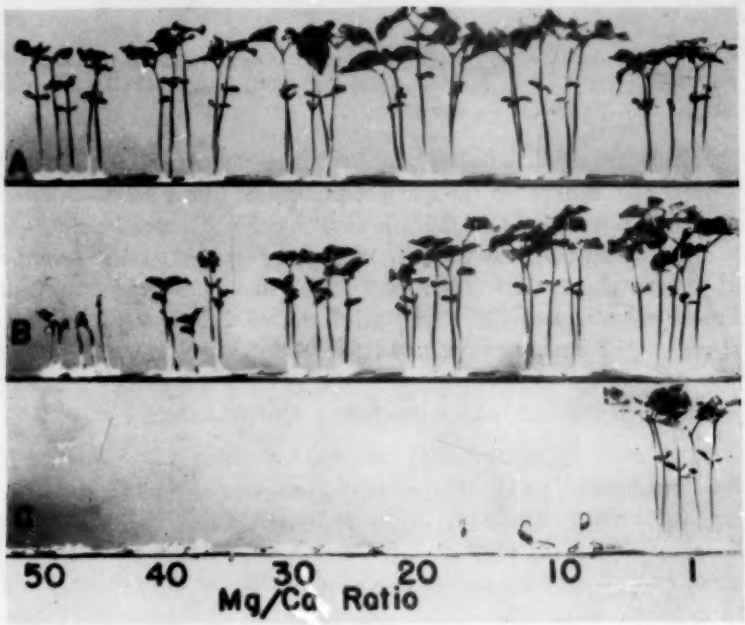

FIG. 5. Responses of the bean plant to the same series of Mg/Ca ratios, but in three different total concentrations of salts. Seven days after planting. Rows A, B and C contain 0.18, 1.8, and 18 ml. of molar $Ca(NO_3)_2$ per liter, respectively.

(15) report that the Mg/Ca ratio must be 2, or less, to avoid magnesium injury. It is of interest that a Mg/Ca ratio of 1.0 was not as favorable for the growth of bean plants as the higher ratios. TOTTINGHAM (17) has reported that high concentrations of $Ca(NO_3)_2$, in a series of solutions with two per cent. (8.15 atm.) concentrations, produced characteristic and serious injury to the tops of wheat plants.

TRELEASE [illegible] TRELEASE (18) state that magnesium injury to wheat, while mainly controlled by the Mg/Ca ratio, appears to be influenced by the proportions and concentrations of other materials present in the culture solution, as well as by the total concentration of the solution and possibly also by climatic conditions. They also report that, between the limits of one and 50 of this ratio, the dry weight of tops of wheat plants is inversely proportional to the log of the Mg/Ca ratio. TRUE and BARTLETT (19) found that absorption by pea roots is greatest in equimolecular proportions of calcium and magnesium nitrates, but that root growth is equally good in any solution which does not contain less than one molecule of calcium to nine of magnesium. In the more dilute solutions, an even lower ratio of Ca/Mg, 1:99, overcame the effects of magnesium alone. GARNER, *et al.* (5), state that it is difficult to distinguish between magnesium toxicity symptoms and minus-calcium symptoms. They found that, in the case of tobacco, there must be present in the leaf 0.25 per cent. of magnesium and 1.0 per cent. of calcium to prevent deficiency symptoms of these cations.

BARBIER (1), working with maize, oats, onion, spinach, and tomato reported that the uptake of an element is a function of its concentration, and concluded that antagonism is relatively unimportant and should not be exaggerated. Similarly, in studies with corn, BECKENBACH, WADLEIGH, and SHIVE (2) observed that none of the three ratios—Ca/Mg, K/Mg, or K/Ca—showed any significance.

This study indicates that there is no well-defined Ca/Mg "ratio" that is optimal for growth over a range of total concentrations of salts, and that it is a matter of interrelationships among calcium, magnesium, and other ions of the solution.

INTERRELATIONSHIP OF CALCIUM AND POTASSIUM

In another series, similar to the Mg/Ca series, calcium and potassium were varied, while $MgSO_4$ was constant at 9 ml. of molar salt per liter of solution. Calcium nitrate was used at three levels of nutrition, just as in the previous experiment, and the two potassium salts (KNO_3 and KH_2PO_4) were varied to produce K/Ca ratios of 200, 150, 100, 50, 10, and 1. Within 7 days, the appearance of the plants was as shown in figure 6. Rows B and C show growth responses that were apparently associated with concentration, *i.e.*, total concentrations become less from left to right. There was no magnesium burning

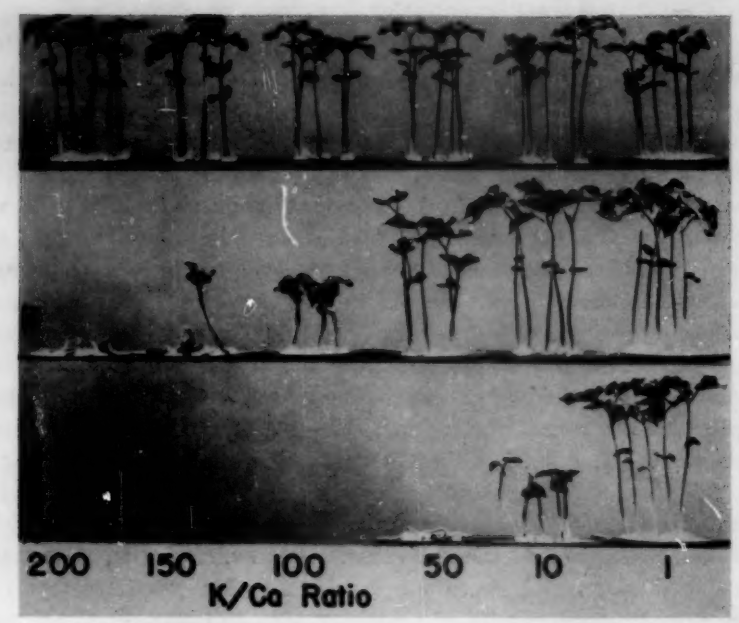

FIG. 6. Responses of the bean plant to the same series of K/Ca ratios, but in three different total concentrations of salts. Seven days after planting. Rows A, B, and C, contain 0.18, 1.8, and 18ml. of molar $Ca(NO_3)_2$ per liter, respectively.

in either row B or C. In row B there was no burning, and the ratio of Mg/Ca was only five. In row A the plants were of similar heights and growth-form, but all of the plants in this row were severely burned owing to the high Mg/Ca ratio of fifty.

According to MCCALLA and WOODFORD (9), limiting the amount of one element in a nutrient brings about an increase in absorption of another ion of the same sign, or a decrease in absorption of all ions of the opposite sign. Limitation of potassium is accompanied by an increase in the absorption of both calcium and magnesium. Limitation of calcium results in a relative increase in the absorption of both potassium and magnesium, with the increase in magnesium being considerably the greater.

Thus it seems that calcium plays an important rôle within the cells and in the so-called physiological balance of nutrient solutions by overcoming, within certain limits, the toxic effects of too high a concentration of any other cation. This interrelation of the various cations is not a static condition; it is dynamic and continually varying—depending upon the relative concentrations of the cations present. These interrelations of cations are presumably effective in part through their action on the colloidal properties of protoplasm.

COMPARISON OF THE A+ AND A– SOLUTIONS WITH THE B+ AND B– SOLUTIONS

Calcium deficiency affects the terminal bud, leaves, and roots of the bean plant in much the same way as reported for tobacco by MCMURTREY (**10**).

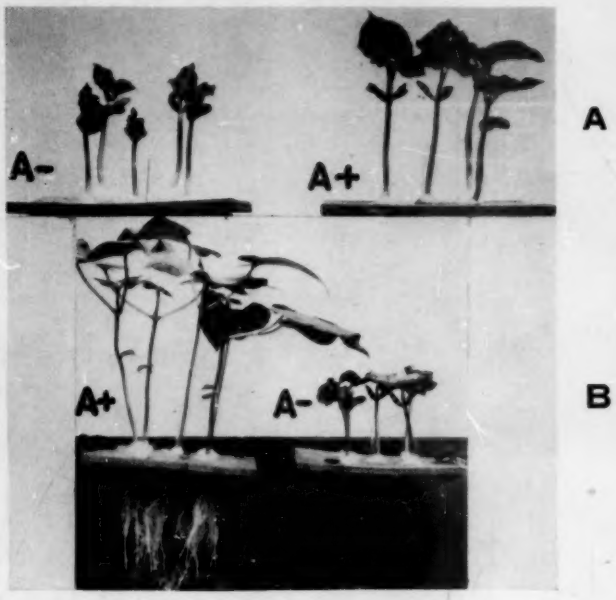

FIG. 7. Growth of bean plants in the A+ and A– solutions. A, three, and B, seven days after planting.

Plants growing in the A– solution (fig. 7) are short (stunted) with small, wrinkled leaves. They die within six to eight days from the time of transfer. The dry weight (fig. 9) and total nitrogen (fig. 13) do not increase from the second to the sixth day. Ash constituents (fig. 10) continue to enter these plants, but nitrates do not. This is in accordance with the findings of NIGHTINGALE, *et al.* (**12**), for tomato. The calcium content (fig. 14) increases slightly and then it decreases. The fact that the roots of these plants contain relatively large amounts of calcium indicates that the calcium, although entering in very small amounts, is bound in the roots In general the dry weight (fig. 11), ash (fig. 12), nitrogen (fig. 15), and calcium (fig. 16) decrease only slightly in the cotyledons of plants growing in the A– solution.

On the other hand, in the B– solution (fig. 8) the dry weight (fig. 9), ash (fig. 10), and total nitrogen of the plants (fig. 13) increase markedly until after the eighth day. The plants live for two weeks or more from the time of transfer, are taller, have larger leaves, and wrinkling, or necrosis, of the

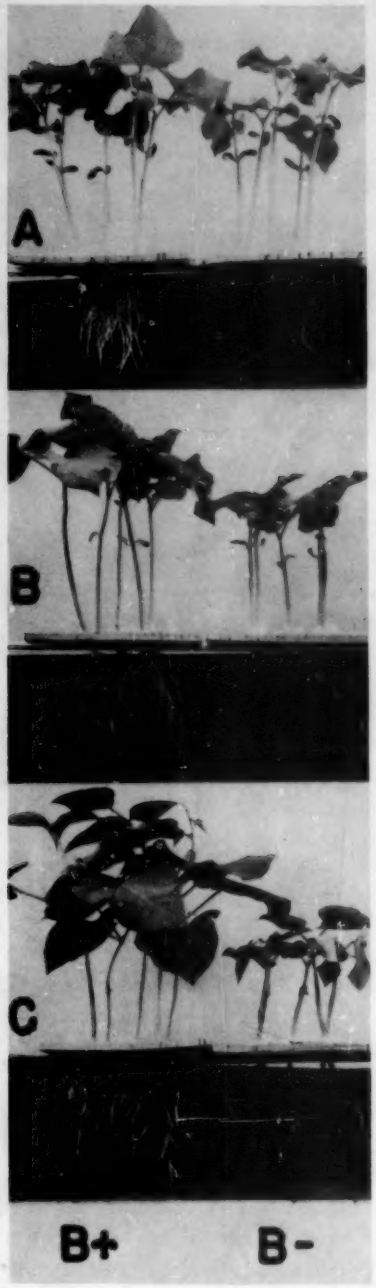

FIG. 8. Growth of bean plants in the B+ and B– solutions. A, four, B, eight, and C, twelve days after planting.

leaves is less pronounced or nearly absent. Ash and total nitrogen increase at approximately the same rate. Despite the fact that the B– solution is a minus-calcium solution, nitrates are absorbed to as great an extent as are the ash constituents. This is in contradiction to the statement by NIGHTINGALE, *et al.* (12), that minus-calcium plants cannot absorb nitrates. It is true that they do not do so in the A– solution, but they do in the B– solution. It seems likely, therefore, that the composition of the A– solution is such that the unbalance in the absence of calcium is responsible for the nonabsorption of nitrates. As mentioned earlier in this paper, the amount of magnesium

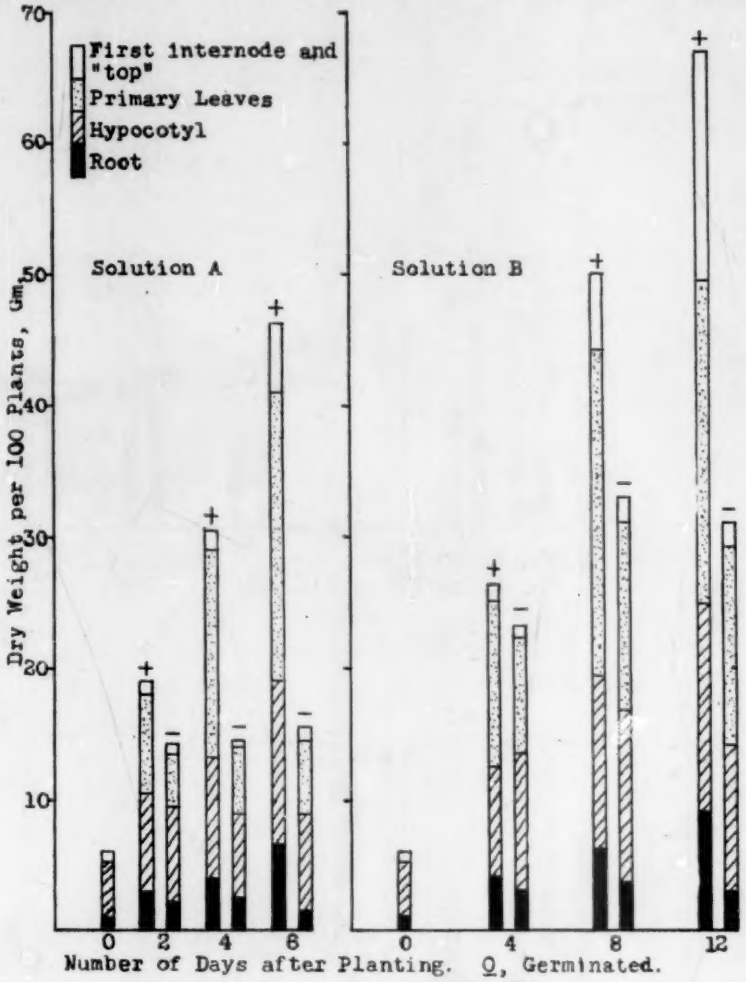

FIG. 9. Dry weight of bean plants.

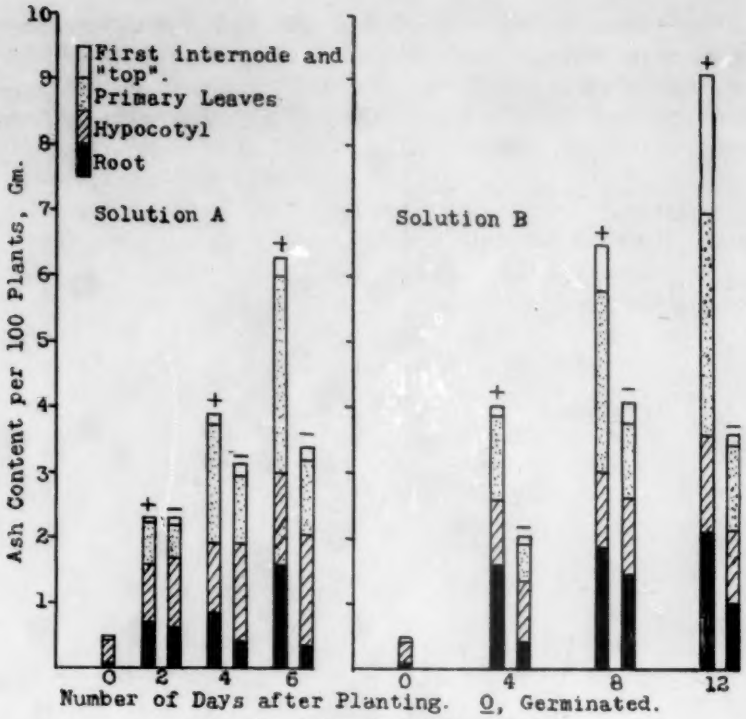

FIG. 10. Ash content of bean plants.

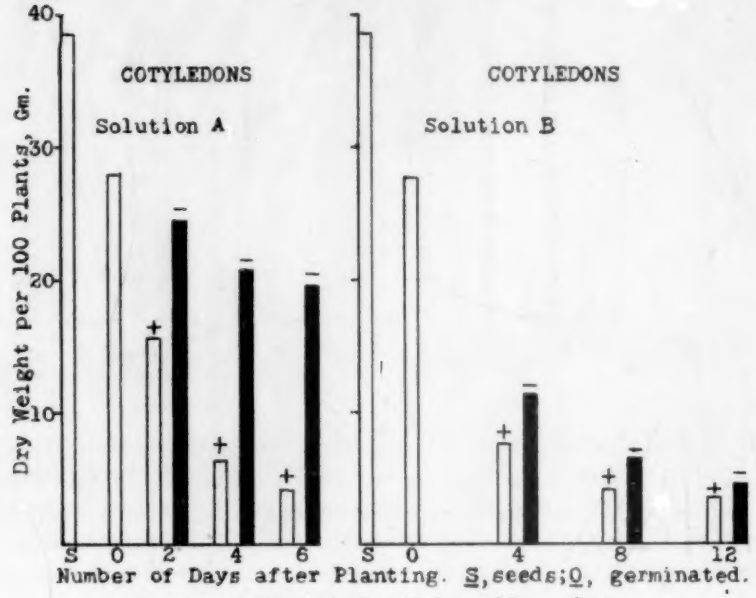

FIG. 11. Dry weight of cotyledons of bean plants.

appears to be one of the main disturbing factors in the A– solution. Inasmuch as the nitrogen content of plants in the B– solution increased markedly, while the calcium content remained more or less constant and low, there is apparently not necessarily a close relationship between calcium and nitrogen contents, as proposed by PARKER and TRUOG (**14**). There may be a correlation, however, between the utilization of nitrogen and the content of calcium. The latter relationship was not determined in this study, but ERMAKOV (**4**) and BURRELL (**3**) have reported that, associated with calcium deficiency, there is an accumulation of nitrates in leaves owing to the fact that nitric nitrogen is not assimilated and converted to organic forms of nitrogen.

In the B– solution, the dry weight (fig. 11), ash (fig. 12), and nitrogen (fig. 15) of the cotyledons decrease to about the same amounts and at the same rate as in the plants of the B+ solution. The calcium content (fig. 16) of cotyledons increases in plants growing in the plus-calcium solutions, and tends to remain more or less constant in the A– or B– solutions. In the absence of sufficient calcium in the nutrient solution, the reserve calcium of the cotyledons is apparently unavailable.

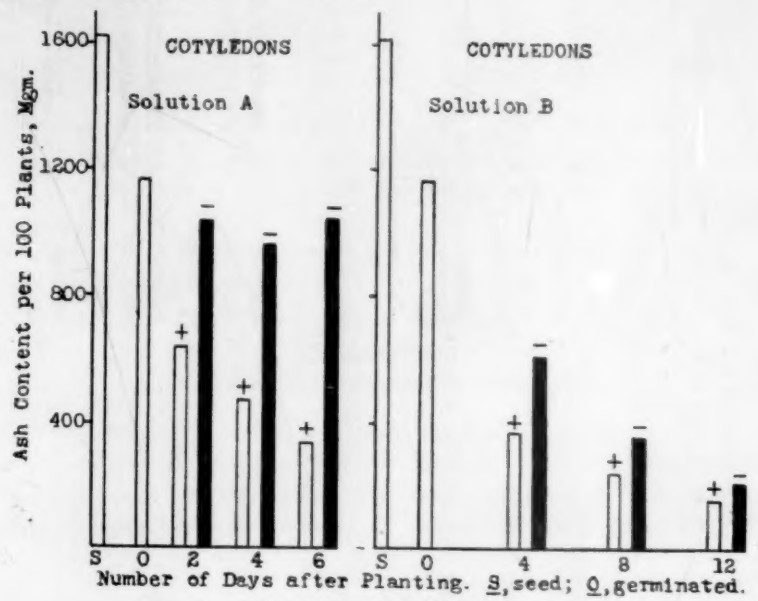

FIG. 12. Ash content of cotyledons of bean plants.

The hypocotyls of plants growing in the A– solution contain more ash than the hypocotyls of plants in the A+ solution, as shown in figure 10. On the other hand, in the B– solution in which nitrates are readily absorbed, the hypocotyls of plants growing in that solution contain more nitrogen (fig. 13) than the hypocotyls of plants in the B+ solution. The reason for this

directional shift of ash and nitrogen to the hypocotyls is unknown, but it is of interest that a similar shift of such materials occurs when the cut surfaces of bean hypocotyls are treated with indoleacetic acid, as reported by STUART

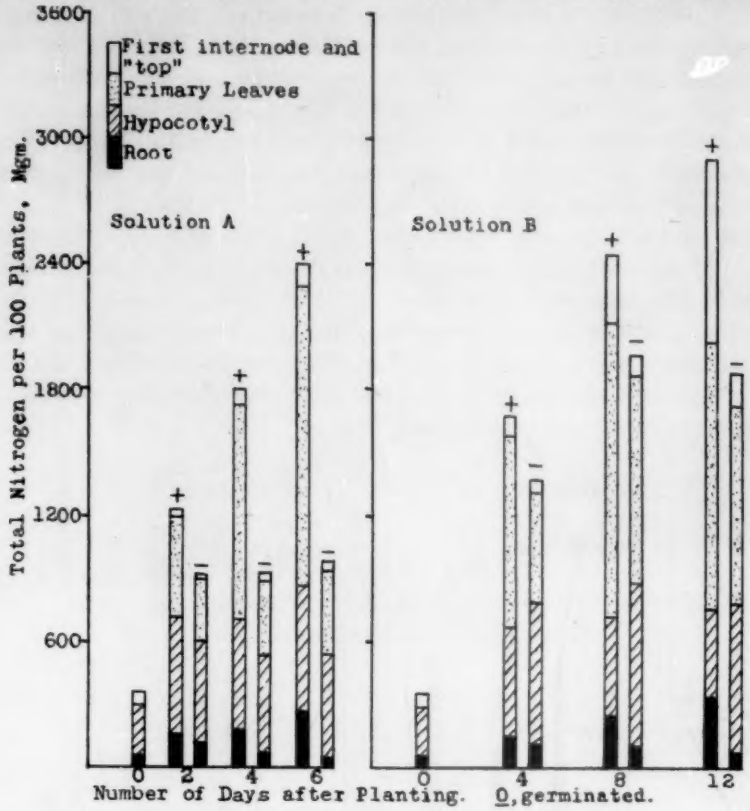

FIG. 13. Total nitrogen content of bean plants.

(**16**). In the response of the bean plant to a deficiency of calcium, including the degeneration of the original root system, there is a tendency toward the production of adventitious roots, especially near the base of the hypocotyl. Such roots do not reach more than one or two millimeters in length.

GROWTH RESPONSES FOLLOWING TRANSFER OF PLANTS BOTH FROM THE B+ TO THE B− SOLUTION AND FROM THE B− TO THE B+ SOLUTION

Each day, for 12 days after planting, plants were transferred from the B− to the B+ solution; plants were similarly transferred from the B+ to the B− solution. As shown in figure 17, the earlier that B− plants were placed on B+ nutrient, the more completely did they resemble B+ plants, and the better was their recovery in general. If transferred between the twelfth and

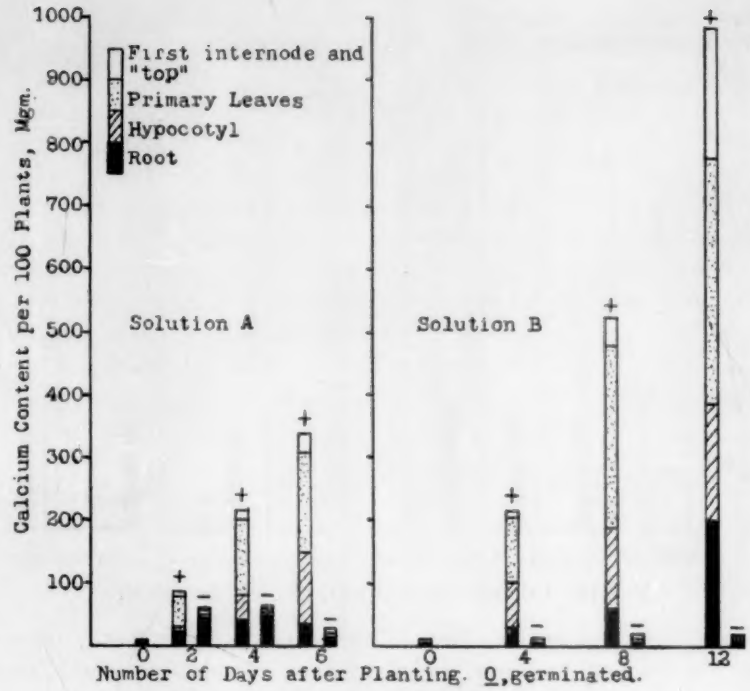

FIG. 14. Calcium content of bean plants.

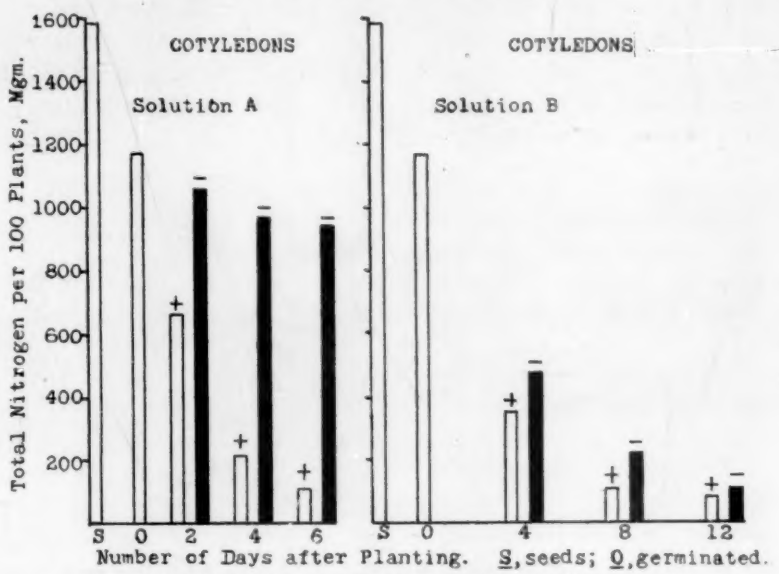

FIG. 15. Total nitrogen content of cotyledons of bean plants.

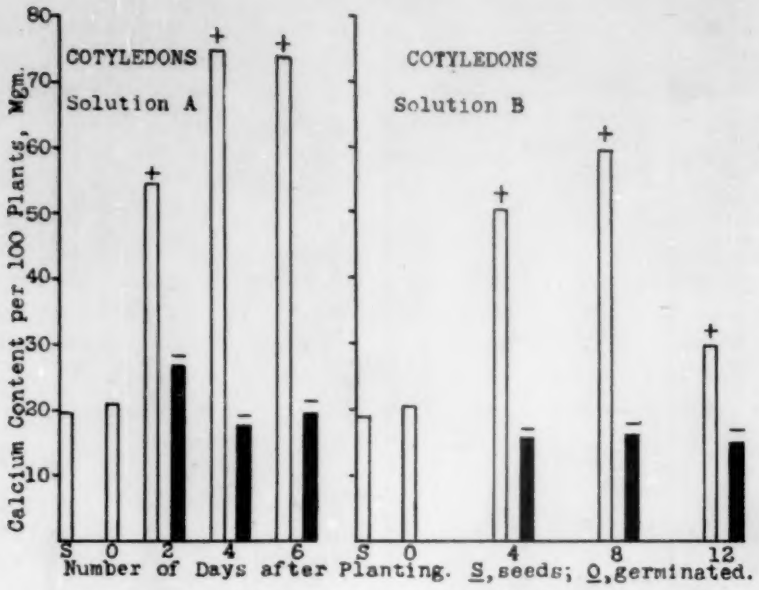

FIG. 16. Calcium content of cotyledons of bean plants.

fourteenth days, the axillary buds of the primary leaves finally grew out, but the terminal bud was dead, as judged from its appearance and from the fact that it did not grow. It seems probable that the terminal bud was originally the most active of the three buds, and that the absence of calcium affected its metabolism first, causing its death before that of the axillary buds. The original roots were dead on the B– plants transferred after six days, but new adventitious roots arose from the bases of the hypocotyls up to and including at least the twelfth day.

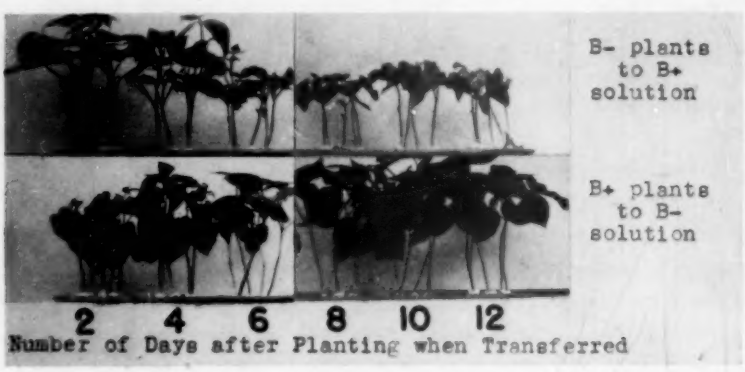

FIG. 17. Response of bean plants after transfer from B– to B+, and from B+ to B– solution. Photograph taken on 13th day after day of planting.

When B+ plants were transferred to B– solution at similar intervals, root growth soon ceased, especially in those B+ plants transferred early into B– solution. Within 10 to 12 days after the second- and fourth-day transfers had been made, the petioles of the primary leaves became weak and the leaves pendant (fig. 17). The cause of this response is unknown, but it approximated the symptoms of high potassium or high sodium. During the first four days after planting, there was a large increase in the amount of ash (fig. 10). In the absence of calcium, apparently relatively greater amounts of other cations are absorbed. With limitation of calcium, McCALLA and WOODFORD (9) observed a relative increase in the absorption of both potassium and magnesium, with the increase in magnesium being considerably the greater. In the B– solution, the total magnesium content is low, but, in this instance, relatively greater amounts of sodium may have entered; the latter element acts, within the plant, much the same as does potassium.

Summary

1. Responses of the bean plant to various concentrations of single salts are reported.

2. From the results obtained in the single-salt studies, various two-salt combinations were made, and the responses of the plants growing therein are reported.

3. On the basis of those and other studies, plus- and minus-calcium solutions (solutions B+ and B–) especially adapted to bean were devised in which there is no apparent excess or deficiency of essential ions other than calcium.

4. The responses of bean plants to two types of solutions were studied, *viz.*, (a) NIGHTINGALE'S, *et al.*, plus- and minus-calcium solutions (solutions A+ and A–), and (b) plus- and minus-calcium solutions adapted to bean (solutions B+ and B–).

5. Bean plants growing in the A– solution continue to absorb ash constituents, but they do not absorb nitrates. This is in accordance with the findings of NIGHTINGALE, *et al.*, for tomato.

6. Plants growing in the B– solution do, however, absorb appreciable quantities of nitrates, and the increases in dry weight, total nitrogen, and ash of these plants are parallel.

7. Dry weight, total nitrogen, and ash of the cotyledons from plants growing in the B– solution decrease markedly in amount, while those fractions decrease only very slightly when the plants are grown in the A– solution.

8. Bean plants, with cotyledons intact, live approximately six days in the A– solution, and twelve days in the B– solution.

9. There appear to be no ratios, *e.g.*, Ca/Mg or Ca/K, that are optimal for growth, over a range of concentrations of salts.

The writer is indebted to members of the Department of Botany of the University of Chicago for helpful suggestions made during the course of this study.

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METABOLIC PROCESSES OF POTATO DISCS UNDER CONDITIONS CONDUCIVE TO SALT ACCUMULATION[1]

F. C. Steward and G. Preston

(with nine figures)

Introduction

Modern investigations (**11, 34**) show that vital activity plays an essential part in salt accumulation by plants. The ability to accumulate[2] demands properties peculiar to cells which are still able to grow and the rate at which these are utilized is causally related to the intensity of aerobic respiration. Salt accumulation is not merely the result of equilibria at membrane surfaces but demands some "dynamic machinery" in cells—to use a happy phrase which received prominence in a recent symposium. A similar concept is implied by the oft repeated assertion that during salt accumulation work is done and energy exchanges are involved. The identification of the "dynamic machinery" and the mechanism by which the energy stored in substances of high energy content is ultimately utilized in salt accumulation is one of the most challenging problems of modern plant physiology.

The earlier work, done on Nitella, Elodea, discs cut from storage organs, and roots, focussed attention upon those biochemical processes in the accumulating cells which involve carbohydrates and suggested that these represent an important part of the machinery of accumulation. In a brief review of available evidence (**33**) it was concluded that the biochemistry of salt absorption represented a virgin field for investigation and this inspired much of the work to be described in this and ensuing papers.

The relationship between salt accumulation and metabolism has been most investigated with reference to respiration as measured by carbon dioxide output. The conclusion to be drawn (**11, 33, 34**) is that the relation between salt uptake and respiration is not a simple and direct one; it concerns the aerobic phases of the respiratory process but it is the oxidation which these involve, rather than the production of carbon dioxide *per se*, which is causally concerned in salt accumulation. The ultimate connection between respiration and salt accumulation must therefore involve some aspect of the oxidative breakdown of sugar—the ultimate source of carbon substrates—other than the mere evolution of the carbon dioxide which is finally produced. The energy thus released must be involved; not only because it supplies the relatively small amount of energy necessary for the actual process of salt uptake, (a process which tends to increase the free

[1] This is the first of a group of papers on the biochemistry of salt absorption.

[2] Especially that most general type of accumulation which involves the simultaneous uptake of anion and cation and which has been termed "primary absorption." Certain absorption processes in which metabolism is not involved are not in question here.

energy of the system concerned) but also because it maintains the cells at that general level of metabolic activity which is an invariable characteristic of systems capable of a high degree of salt accumulation. Hence the biochemistry of salt accumulation entails an account of the fate of the carbohydrates metabolized during salt accumulation—an account which must ultimately be tested by a balance sheet of carbohydrates and their energy values. This aspect will be considered in a subsequent paper (**36**).

The relation between salt uptake and metabolism cannot, however, be restricted even to the processes of carbohydrate metabolism for it demands also a general picture of all the metabolic processes of cells engaged in salt accumulation. Only by the effect of the variables which determine accumulation upon these metabolic processes can those which are causally connected with salt uptake be recognized. It has been emphasized (**37**) that the conditions conducive to salt accumulation should also cause not only an enhanced respiration (oxygen uptake and carbon dioxide production), but also protein synthesis at the expense of soluble nitrogen reserves of the storage tissue. These processes should also be accompanied by a considerable evolution of heat; in fact, by all those processes which eventually lead to cell division and are involved in a recrudescence of growth in cells which were previously dormant in the storage organ. At that time much had to be inferred from evidence cited from other investigations as actual quantitative measurements of some of the metabolic processes described had not then been made.

The new evidence establishes the reality of some of the metabolic processes of thin, aerated potato discs which was hitherto presumed upon less direct evidence. This paper indicates the scope of the survey of metabolism which the general problem demands and deals with methods suitable for this type of investigation. Justification for the experiments to be described does not rest alone upon the more complete account they give of the metabolites of cells engaged in salt accumulation; they may also be scrutinized from another and still more general standpoint. The true nature of the relation between respiration and concomitant metabolic processes is as obscure as that which respiration bears to salt accumulation. Since the experiments to be described in this and subsequent papers record the respiration and metabolism of tissue subject to rigorous control, the data are relevant to the more general problem. The ultimate objective is a synthesis of results into a scheme which explains the mutual relations of all those vital processes which are dependent upon respiration for energy. The experiments to be described in this and following papers contribute to this end.

For the problem as thus envisaged the cut discs of storage tissue used in the earlier work have special advantages. The experimental technique (**31**) devised for the investigation of respiration and salt uptake is also necessary for the investigation of metabolism since the variables thus controlled (oxygen supply, temperature, and disc thickness) also control metabolism. The

amount of tissue used in each culture vessel (not exceeding 60 standard discs of 0.75 mm. thickness and a total weight of 45 gm. or its equivalent surface) is adequate for detailed biochemical examination and, being random sampled from a large batch of discs, the variability between replicate cultures can be reduced until it becomes negligible for most purposes. The general effect of oxygen concentration, temperature, and disc thickness upon respiration and salt uptake of potato discs is known and this information may be used when arbitrary values must be assigned to these variables. Previous work has localized the most active cells which contribute the bulk of the total respiration of the discs to a thin, hollow, cylindrical shell of tissue—the dimensions of which are known for standard conditions—which is situated at the surface of the disc. The technique which has evaluated the respiration rate, or the salt accumulation, of cells in given situations within the disc could also be applied to other metabolic processes. Hence the physiological behavior of thin discs of storage tissue need no longer be interpreted on the mistaken assumption that all of the cells of the disc are identical; on the contrary this type of experimental material has its greatest utility when it is recognized that the degree of activity in the cells is determined by their position within the disc. Thus the correct emphasis may be given to the very active cells which constitute that thin shell of tissue in which the properties of growth and active metabolism reside. Relative to the larger problem which involves the relation between respiration and other metabolic processes there is special reason for selecting experimental material in which cells retain the capacity for growth and cell division and also environmental conditions which permit these properties to be exercised. Senescent cells which have lost the properties in question clearly lack some fundamental metabolic property and exhibit a relationship between respiration and oxygen concentration which differs from that encountered in cells which are still able to grow. For example, contrast the effects of oxygen concentration on the respiration of potato discs (**32**), carrot and artichoke (**35**), potato roots (**35**), barley roots (**11**), and various seedlings (**15, 16**) with the effects of oxygen on the respiration of apples (**21**), tomato fruits (**10**), etc. The relationship of respiration to metabolism, therefore, may be different in kind in senescent and growing cells and there is every reason to believe that the latter constitute the most general case for the investigation of such problems.

Experimental methods and results

Composition of potato discs and basis for the expression of analytical results

The basis for the calculation of the results of analyses which portray the course of metabolism should be some quantity which is not subject to change.

Neither the final fresh nor dry weight of the rinsed, surface-dried discs fulfil this requirement. The initial fresh weight of a known number of standard discs is free from objection since the initial composition of replicate batches of discs random sampled from a large stock can be accurately reproduced. Variation in the fresh weight of replicate batches of standard potato discs, whether cut at the same time or not, is small if the stock of tubers is uniform (table I).

TABLE I

MEAN FRESH WEIGHT OF 50 DISCS

No. of replicates	Mean fresh weight	Standard deviation	Standard deviation as percentage of mean
	gm.	*gm.*	%
8	38.5	0.49	1.27
8	38.2	0.32	0.85

The uniformity in size and fresh weight of the discs cut and then washed 24 hours in running tap water does not conceal a much greater disparity in their composition as shown by the data of table II.

TABLE II

THE DRY WEIGHT AND CARBON CONTENT OF POTATO DISCS

No. of replicates	Fresh wt.	Dry wt.			Carbon content		
	Mean	Mean	Standard deviation* as percentage of mean	As percentage of fresh wt.	Mean	Standard deviation* as percentage of mean	As percentage of fresh wt.
	gm.	*gm.*	%	%	*gm.*	*gm.*	%
4	27.8	4.22	1.73	15.1	1.62	5.57	38.6

* Standard deviation of the mean as a percentage of the mean = ½ this amount.

Table III shows the total sugar and nitrogen content of standard, washed discs cut from the same stock at various times during one month of the rest period. It will be seen that both the differences between parallel samples (series C and D) and between the different time series (series A, B, C, D) are small relative to the order of the effects to be described later. Where comparisons between experiments not run concurrently are made in this work the experiments were carried out within the shortest possible time to eliminate effects due to changes during storage.

Even where known varietal differences and great disparity in the soil

TABLE III

SUGAR AND NITROGEN CONTENT OF STANDARD DISCS

SERIES	SUGAR PER GRAM FRESH WEIGHT	TOTAL NITROGEN PER GRAM FRESH WEIGHT	FRESH WEIGHT PER DISC
	mg.	*mg.*	*gm.*
A	3.53	2.06	7.43
B	2.74	2.05	7.56
C	3.15	2.05	7.91
	3.24	2.08	7.90
D	2.99	2.06	7.86
	3.20	2.05	7.94

TABLE IV

COMPOSITION OF DIFFERENT STOCKS OF POTATO DISCS

STOCK	DRY WEIGHT PER 45 GRAMS FRESH WEIGHT	STARCH PER 45 GRAMS FRESH WEIGHT	SUGAR PER 45 GRAMS FRESH WEIGHT	PROTEIN N PER 45 GRAMS FRESH WEIGHT	SOLUBLE N PER 45 GRAMS FRESH WEIGHT
	gm.	*gm.*	*gm.*	*mg.*	*mg.*
1934	6.40	4.28	0.22	37.8	59.0
1935	(a) 5.78	4.04	0.16	34.6	56.7
	(b) 5.74	3.79	0.12	33.2	59.4
	(c) 5.43	4.03	0.14	30.6	61.6

conditions are involved the principal organic constituents of standard potato discs are remarkably constant. This is well shown by the analysis of the two principal stocks of tubers used in the work to be described in this and a later paper. The one (1934, experiments at Berkeley, California) was a variety grown in the Delta soils of California which are rich in salts. The other (1935) represents King Edward tubers grown in Lincolnshire, England; samples A, B, and C were taken at different times during the storage period when experiments were made. Despite the evident uniformity of the composition of potato tubers the comparisons were mainly made between parallel treatments applied to discs random sampled from a uniform batch cut at one time.

METABOLIC PROCESSES INVESTIGATED AND METHODS USED

The processes chosen for investigation and the technique adopted are outlined below.

CHANGE IN FRESH WEIGHT.—As in earlier work, this very valuable index of the behavior of the tissue was obtained by weighing the discs after they

were rinsed, and the surface dried twice with blotting paper. When the final sample was subdivided for analysis the fresh weight of the various aliquot parts was recorded.

CHANGE IN DRY WEIGHT.—Dried samples were used for some of the determinations (*e.g.*, calorific value). The dry weights were determined as follows. The fresh tissue was heated *in vacuo* for one hour at 100° C. to destroy enzymes. Constant weights were obtained after 36 hours drying *in vacuo* at 60° C. The dried ground tissue was always redried *in vacuo* at 60° C. before aliquot parts were withdrawn for analysis.

RESPIRATION.—Methods by which the respiration of potato discs immersed in aerated salt solutions can be measured have been fully described (**31**). Whenever the total carbon dioxide—in contrast to the average rate during a brief period—was required, the residual external solution was acidified, aspirated, and the carbon dioxide yield determined. This correction attains importance in nitrate and calcium solutions.

When effects of salts on respiration are involved the interaction between the salt effect and time is often of importance. Hence methods which yield data at shorter time periods were desired and for this purpose the respiration determination was modified as follows.

TYPES OF ABSORPTION TOWER

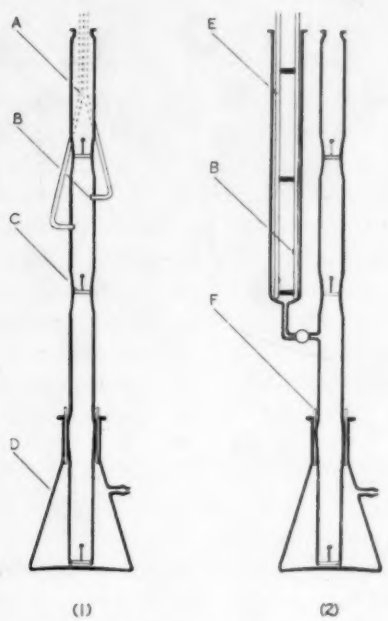

FIG. 1. Types of absorption tower for use with conductivity methods.

Into the modified Reiset absorption towers (fig. 1), of the type previously described (**31**) platinized-platinum electrodes [B, (1) fig. 1] with an area of 0.18 cm.^{2} were inserted at a level between the two upper perforated silver discs. To increase electrical resistance in contact with a maximum concentration of 1.0 M caustic soda the electrodes were placed 2.0 cm. apart horizontally and 3.0 cm. apart in the vertical direction as in the method of NEWTON (**20**). External contact was made by means of tubes containing mercury [A, (1) fig. 1]. The gas stream was stopped during readings and the contents of the tower previously mixed by raising and lowering the liquid. It is sometimes convenient to insert before the tower a T-piece and a capillary resistance which can be opened at will, by a screw clip, to air. In this way the gas stream may be temporarily diverted and by operation of screw clips the absorption liquid can be rapidly raised and lowered. Others have shown (**4, 20**) that over a wide range the decrease in conductivity of a caustic alkali solution bears a linear relation to the carbon dioxide absorbed. The towers in question were calibrated for a fixed volume of alkali by carbon dioxide generated from known amounts of bicarbonate solution. Specimen curves for two such towers[3] are shown (fig. 2). The equation to the line shown was

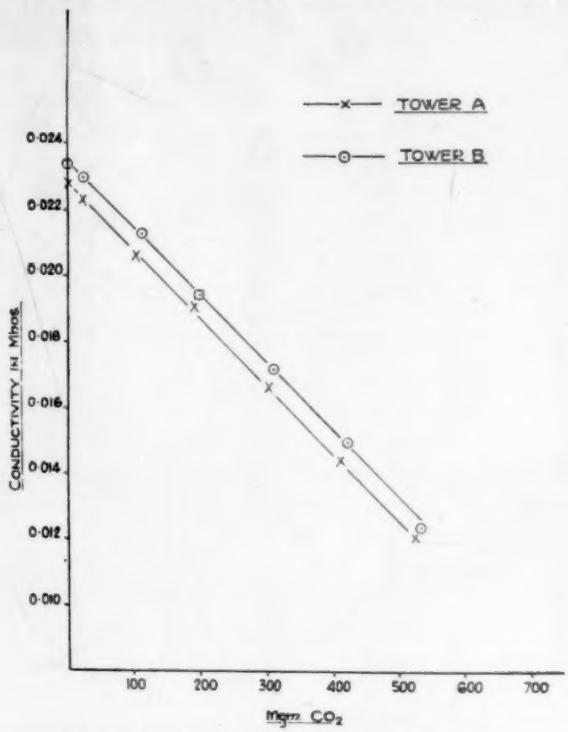

FIG. 2. Calibration curves of absorption towers.

[3] Charged with 50 ml. of MNaOH plus 100 ml. of water.

found by the method of least squares. For a constant volume of absorbing liquid (150 ml.), the constant of slope in the equation is characteristic of the tower and is unaffected within a narrow range, by the total concentration of alkali. Respiration rates were most conveniently expressed as mg. carbon dioxide per gm. initial fresh weight per hour.

Figure 1, (2) shows another modification which has the advantage that several towers may be used with one calibrated electrode and the platinized surfaces are only in intermittent contact with the alkali. A side arm communicates with the tower via a stopcock. During absorption the side arm is closed by a rubber stopper and the stopcock is shut. A rigid pattern of dipping electrode (E), with platinized platinum plates (B) is immersed in the absorbent which, for the purpose of reading, was transferred to the side arm by appropriate manipulation of the gas stream and stopcocks. Mixing was effected by raising and lowering the liquid as in the type (1). During

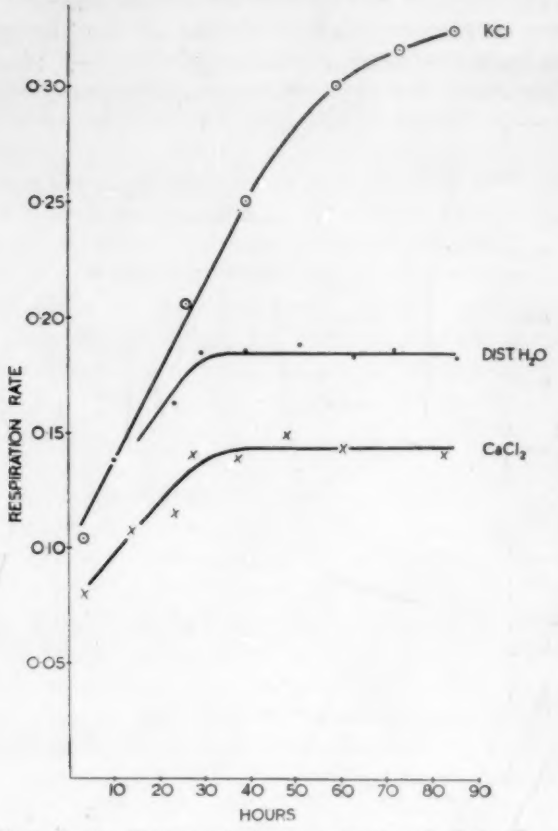

FIG. 3. Effect of salts (0.050 equivalents per liter) and of time on the respiration of potato discs at 23° C.

mixing the apparatus beyond the tower was isolated by a screw clip and the pressure before the tower released, by the slow passage of the accumulated gas to air, through capillary resistance again attached as a side arm by a T-piece.

Effect of salts and of time on the respiration of potato discs

Using the above method for carbon dioxide determination the effect of time on respiration was determined for parallel cultures of 50 discs (average thickness approximately 0.75 mm.) placed in distilled water, 0.05 equivalent KCl, and 0.05 equivalent $CaCl_2$ respectively. Figure 3 shows clearly that after about 25 to 30 hours, during which a rising respiration rate obtained as the tissue became adjusted to the conditions of temperature and aeration, a steady level of respiration (0.186 mg. CO_2 per gm. per hour) was attained. Similar tissue in 0.05 equivalents potassium chloride per liter, and at the same oxygen concentration and temperature, finally attained a respiration rate 1.75 times as great as that in distilled water; whereas in calcium chloride of the same equivalent strength, the steady level attained was at 0.144 mg. CO_2 per gm. per hour. The behavior in time of discs in distilled water has long been a familiar feature of this work (**32, 37**) and, since all the factors affecting respiration were not known, it has been recognized that the plateau in the time curve was due to some factor, other than oxygen concentration, which was in relative minimum (**32**). It is clear from these curves that the essential factors concerned, which regulate the pace of the respiration of the tissue, are conditioned by the salt concentration in the external solution. The opposed action of potassium and calcium salts with a common anion indicates that it is the cations which are the causal agencies. The elucidation of this effect must await description of the other detailed metabolic effects caused by the salt treatment.

Change in calorific value

The energy value of the processes of metabolism should be measured when the ability of cells to carry out processes which require energy is in question. Ideally this requires the measurement of both the total energy lost by the metabolizing cells in the form of heat and also of the change in the calorific value of the cells. Assuming that the cells do work the tissue should decrease in calorific value in excess of the energy which is lost merely in the form of heat and, if these measurements proved to be possible, a direct measurement of the energy converted into work by the tissue might be made. At the outset it must be recognized that this ideal is as yet unattainable but the experiments here described represent a first contribution towards this ultimate objective.

It is a well known fact that at a cut potato surface heat is produced in a quantity sufficient to raise the temperature of the superficial cells above that

of tissue which is deep seated and thus less active (**27**). Assuming that the bulk of the carbon dioxide production of these potato discs arises from the aerobic breakdown of sugar and that the energy value of this reaction appears in the form of heat in accordance with the equation

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 674{,}000 \text{ cal.}$$

then at a rate of respiration of 0.20 mg. CO_2 per gm. per hour—a rate which may be long maintained at 23° C.—one might anticipate a continuous heat production of the order of 22 cal. per hour for cultures of 60 discs (45 gm.). Thus stated the measurement of the respiration of potato discs by its heat production seems to be within the scope of the refined heat measurements which have been used in other types of investigations. The measurement of the heat production of nerve by DOWNING, GERARD, and HILL (**7**) involved the measurement of heat production of the order of 10×10^{-6} cal. per gm. per second. The differential calorimeter of RANDALL and ROSSINI (**25**), equipped with multiple junction thermocouples and devices which measure a temperature rise as small as $21/5 \times 10^{-6}$ °C. in a calorimeter of heat capacity of the order of 65 cal. per degree, has been applied by FIFE (**9**) in an intensive investigation of the heat production and respiration of Azotobacter. FIFE was able to show that the heat produced by Azotobacter was exactly equal to the amount to be expected from the carbon dioxide released. In other words, within the limits of error, *all* of the energy released by those respiratory processes which culminate in carbon dioxide output was accounted for as heat and the source of the energy needed by the organism remained obscure. ALGERA (**1**) has used an automatic calorimeter (accurate to 1.4 per cent. on a heat production of 20 cal. per hour), in which the heat production of the organism (*Aspergillus niger*) is compensated by evaporation caused by forcing dry air into the system with a special pump. The change in the calorific value of the solution and the mycelium was measured as well as the heat and carbon dioxide evolved. The data led to the surprising conclusion that the energy derived from the respiration of glucose to carbon dioxide and water is not involved in the synthetic processes of the growing fungus but it is dissipated in the form of heat.

Direct attempts, however, to measure the continuous heat production of potato discs under the conditions conducive to salt accumulation were abandoned for the following reasons. Of necessity the tissue had to be immersed in a relatively large volume of solution and this increased the heat capacity of the culture vessel to such an extent that the most sensitive type of thermal measurement would be necessary. The correction imposed by the heat leak of the apparatus, a correction which even the best of large wide-mouthed Dewar flasks entail, was further complicated by the additional corrections due to the stirring system (the heating effect due to the mechanical stirrer and the conduction due to necessary metal parts) and also to the effect of the rapid air stream used for the supply of oxygen. It became apparent

that on a maintained heat production of the order of 22 cal. per hour, and with a calorimeter with a heat capacity of the order of 2 to 3 kgm. cal., the expected heat production was too near the order of the various corrections incidental to the technique and the apparatus to make this a profitable line of approach, at least in the early stages of the problem.

Attention was therefore confined to the second part of the heat problem, namely, the measurement of the total heat changes as shown by the calorific value of the discs. If the theoretical amount of heat from the conversion of sugar to carbon dioxide and water is to be regarded as lost by the tissue to the surroundings (and the justification for this now rests mainly upon the work of FIFE and ALGERA), then comparison can be made between this quantity and the observed decrease in calorific value. Thus a first picture could be obtained of the energy exchanges involved in the metabolism of potato discs. Whereas the direct measurements of the continuous heat production of the immersed living discs would tax even the most refined technique, preliminary observations showed that the over-all change in calorific value caused by a period of 88.25 hours of metabolism at 23° C. was readily measurable, as shown by the data of table V obtained from discs treated under the standard conditions of the salt accumulation experiments.

These figures, which could be supplemented by many others of a similar nature, serve to demonstrate at once the very considerable magnitude of the heat changes which occur in the discs and also to present the problem that the loss of heat thus observed was *much in excess of that to be anticipated from the measured carbon dioxide production.* It seemed, therefore, that the determination of calorific value could be profitably applied to the problem of salt absorption and metabolism.

The measurements of calorific value in table V, were made using an

TABLE V

CHANGE IN CALORIFIC VALUE DURING METABOLISM OF POTATO DISCS*

SAMPLE	CONDITIONS	FRESH WEIGHT 40 DISCS	VACUUM DRY WEIGHT 40 DISCS	CALORIES PER GRAM DRY WEIGHT	TOTAL CALORIES	CHANGES DURING METABOLISM		
						CALORIES OBSERVED	RESPIRATION	
							CO_2	CALORIES CALCULATED
		gm.	*gm.*	*cal.*	*cal.*	*cal.*	*mg.*	*cal.*
Initial	.....	32.50	5.296	4054	21,480	.....	.....	.....
Final	Distilled water	34.90	4.456	4207	18,750	2730	687.2	1755
Final	0.001 N KBr	35.95	4.539	4176	18,910	2570	730.2	1865

* 40 discs exposed to 2 liters of solution under standard conditions (33) at 23° C. for 88.25 hours. Solutions aerated by washed air at 15 liters per hour.

EMERSON calorimeter (a standard form of gold lined bomb calorimeter) with an oxygen pressure of 20 atmospheres. Thermocouple methods for the determination of the heating and cooling curves of the bath, before and after combustion, were not employed but the BECKMANN thermometer which was used was especially calibrated over the necessary part of the scale. An electrical vibrator activated at the time of each reading, prevented the meniscus from adhering to the walls. The determinations were made on amounts of approximately 1.0 gm. of dried, ground, and uniformly sieved potato tissue. Special attention had to be given in all of this work to the determination of dry weight and the redrying which must precede the removal of aliquot parts of dry matter for all analyses. For calorimetrical work the potato tissue was not pressed into a pellet but was mixed with sufficient magnesium oxide and ignited electrically on a small boat—thus the formation of small incompletely burnt pellets of carbon was prevented. The heat capacity of the calorimeter and bomb (2200 cal.) determined from the combustion of 1.0 gm. of sugar or benzoic acid, included a standard volume of water within the bomb which was sufficient to dissolve soluble products of combustion. The temperature of the water bath which contained the bomb was maintained uniform by a stirrer, the speed and operation of which were rigorously controlled. Special care was taken to procure symmetrical heating and cooling curves. The order of the expected temperature rise could be estimated in the light of experience and the weight of water in the bath and its initial temperature were accurately adjusted so that prior to ignition a smooth heating curve was obtained and finally a smooth cooling curve from a temperature approximately as much above room temperature as the bath temperature at the time of firing was below. These conditions permit the best results from the application of certain empirical constants in the calculation.

Prior to firing, the heating curve of the water bath was obtained by readings every half minute; the time of firing was noted and the course of the ensuing temperature rise and subsequent fall was similarly recorded. The observed maximum temperature increase was corrected for the thermometer error and also by a "radiation correction" calculated from the empirical formula suggested by SCOTT (**29**) in his description of the determination of the calorific value of coal. The calculated correction compensates for the errors due to radiation and the time lag in the response of the water bath. Unburned fuse wire, if any, was weighed and the appropriate correction applied. Under the best conditions combustions which involved the production of approximately 4000 cal. could be repeated on successive samples to within 50 cal.[4] In all cases where values are assigned to tissue samples in this and following papers the figure given is the mean of concordant, replicate determinations and its accuracy is of the order named.

[4] This accuracy was due principally to the care and time expended on these determinations by P. R. STOUT of the division of Plant Nutrition, University of California.

Carbohydrate and nitrogen metabolism

It was essential that the scheme of analysis should reveal the hydrolysis of starch to sugar and its utilization in the metabolism of the discs. Though ultimately desirable, a complete analysis of the various hexose sugars has not yet been attempted; the carbohydrate analyses were confined to the determination of starch and total sugar. Furthermore, to economize material and time it was necessary to make as many determinations as possible upon a single extract. The essential nitrogen and carbohydrate data were therefore obtained by the following scheme:

(1) Potato discs were killed, either by heating for 1 hour at 100° C. followed by drying to constant weight at 60° C. *in vacuo*, or by boiling on a water bath for 20 minutes in excess of alcohol at a final concentration of 70 per cent. by volume.

(2) Complete extraction of either alcohol killed discs or vacuum dry tissues by 70 per cent. alcohol was carried out on an electric water bath with reflux condenser.[5] The accumulated alcohol extracts were evaporated to dryness, the residue dissolved in water and made up to standard volume. On aliquot parts of this solution the total nitrogen (alcohol soluble N) was determined by micro-Kjeldahl methods (**24**) and, after clearing[6] with neutral lead acetate, deleading with oxalic acid (**17**), and acid (3 per cent. HCl) inversion, the total sugar was determined by the Munson-Walker gravimetric method (**6**).

(3) The residue from alcohol extraction was dried, weighed, and used for the determination of alcohol insoluble nitrogen (protein N) and starch. The starch was digested with a salivary extract under toluene, alcohol extracted, the soluble products of hydrolysis inverted, the sugar determined by the Munson-Walker method, and the result calculated in terms of glucose (**2, 3, 23, 42**).

A full record of the fate of the carbohydrates during the metabolism of potato discs will be given in a subsequent paper. It is necessary only to remark here that discs, cut from dormant tubers and washed in running tap water for 24 hours, commonly have a total sugar content of about 3.0 mg. per gm. fresh weight (table III) whereas after 72 hours in aerated distilled water the concentration of sugar increases to 8.0 mg. per gm. This confirms the evidence of starch hydrolysis which can be obtained microscopically.

The scheme needs further comment with reference to the nitrogen determinations. The alcohol-soluble nitrogen will be designated "soluble N"

[5] For special purposes (see under amide-nitrogen) a "cold alcohol extract" is desirable. This was made at about 5° C. in a refrigerator.

[6] In work in which the changes in sugar concentration, rather than absolute values, are involved the clearing technique can be omitted, as the amount of non-sugar material in potato determined by the Munson-Walker method is small (order of 2 per cent. in "final" tissue).

and the alcohol-insoluble nitrogen will be identified as "protein N." This is subject to the reservation that the potato is relatively free from alcohol-soluble proteins and alcohol-insoluble non-protein nitrogen. It was anticipated that during the metabolism of the discs protein synthesis would occur and, therefore, that the alcohol-insoluble fraction would increase at the expense of the alcohol-soluble portion. A test of the recovery of the total nitrogen would be the constant sum of the two fractions. Difficulty was never encountered with the accurate recovery of total nitrogen, mainly because the discs do not lose nitrogen appreciably to aerated solutions.

Since many of the experiments to be described did show protein synthesis by the alcohol method the following evidence is quoted because it confirms that the *changes* in the alcohol-insoluble nitrogen really did measure the change in protein content. In table VI are given the changes in nitrogen

TABLE VI

PROTEIN SYNTHESIS BY POTATO DISCS. COMPARISON OF THE ALCOHOL AND TRICHLORACETIC ACID METHODS

SERIES	SALT SOLUTION	SAMPLE	BY CCl_3COOH METHOD		BY ALCOHOL METHOD	
			PROTEIN N PER GRAM INITIAL FRESH WEIGHT	CHANGE IN PROTEIN N CONTENT	PROTEIN N PER GRAM INITIAL FRESH WEIGHT	CHANGE IN PROTEIN N CONTENT
			mg.	*mg.*	*mg.*	*mg.*
A	.....	Initial	0.66		0.77	
	0.075 equiv. KBr	Final	1.31	+ 0.65	1.42	+ 0.65
B	.....	Initial	0.66		0.74	
	0.075 equiv. $CaBr_2$	Final	0.52	− 0.14	0.64	− 0.09

content of potato tissue which in series A had been exposed for 63.1 hours to aerated KBr solution and in series B had been exposed for 87.3 hours to aerated $CaBr_2$ solution at 23° C. At this stage these treatments need concern us only in that one produced a significant gain of protein and the other a slight loss. The samples were analysed both by the alcohol method and also by the use of trichloracetic acid as a protein precipitant and thus, by comparing the initial and final discs, independent estimates of the change of protein content could be calculated.

It will be seen from the table that the fact of protein synthesis in potato discs exposed to aerated solutions of potassium salts is established. This is not the place to enlarge upon the contrast between the results in the different salt solutions except to note that the effects of the salt on synthesis of protein are parallel to those already described on respiration.

Clearly the different methods yielded almost identical results. The discrepancy, though small, is real and the trichloracetic method yielded protein values which were lower than those by the alcohol method. The estimates of *protein synthesised* were unaffected, however, by the method of analysis (series A). It should be mentioned, however, that further difficulty has been encountered in the analysis of tissue rich in phosphate after absorption in strong phosphate solutions. In this case the alcohol method is unaccountably at fault and yields values which are too low; such difficulties may be overcome by the use of the trichloracetic acid procedure. The method adopted, then, is as follows. The dried, ground tissue is redried at 60° C. *in vacuo*. Aliquot parts of about 50.0 mg. are transferred to 50 ml. pyrex beakers and stirred with 20 ml. of 2.5 per cent. trichloracetic acid for about 15 minutes. The insoluble residue containing protein is then filtered, washed, and transferred on the paper to a Kjeldahl flask in which it is digested in the usual way.

Nature of the compounds concerned in protein synthesis

The reality of protein synthesis in thin aerated potato discs under the standard conditions conducive to salt accumulation can be sufficiently established by reference to the fractions designated "soluble nitrogen" and "protein nitrogen" (table VI, series A). Later papers will be greatly concerned with the effect of different variables upon the process of synthesis. It is, therefore, necessary to specify in more detail the compounds which are involved. The soluble nitrogen fraction is a composite one and includes both amino acids and amides. Free ammonia is a negligible component of the soluble nitrogen except when the tissue has absorbed large amounts of nitrate, or has been so treated during analysis that ammonia arises from the breakdown of relatively unstable soluble nitrogen compounds (amides) which are normal constituents of the potato tissue.

The first point which arises, and one which assumes greater importance in the light of the relationship between respiration and nitrogen metabolism to be discussed later, is the relative contribution of amino acids and amides to the nitrogen used in protein synthesis.

This question may be answered directly by reference to the results of the further fractionation of the soluble nitrogen fraction and, indirectly, by reference to the changes caused in protein synthesis in the titration curve of the sap extracted from the frozen tissue. Before dealing with the type of nitrogen compound concerned in protein synthesis, however, the existence in the actively metabolizing potato discs of unstable nitrogen compounds and their effect upon the fractionation of the "soluble nitrogen" must be appreciated.

Unstable nitrogen compounds.—The soluble nitrogen fraction obtained by alcoholic extraction of the fresh discs contained nitrogen compounds of

very different degrees of stability. It was observed that the evaporated alcoholic extracts contained unexpectedly large amounts of ammonia which can be attributed to "easily hydrolyzable amides" of the kind which VICKERY (**43**) has shown may be hydrolyzed at pH 7.0 by boiling for two hours. The following evidence establishes that unstable amides are real components of potato tissue and also shows their relation to protein synthesis.

Extracts of the soluble nitrogen of potato tissue were made in two ways: a "hot extract" made from the fresh tissue by boiling in 70 per cent. alcohol (pH 5.8 to 6.0), and a "cold 70 per cent. alcoholic extract" made at 5° C. from the dried tissue. The amino-nitrogen and ammonia-nitrogen content of these extracts calculated to the initial fresh weight of the discs (table VII) clearly show that the method of hot extraction released ammonia from some component of the soluble nitrogen fraction which, though stable in cold 70 per cent. alcohol, decomposed on boiling for several hours.

Table VII shows that the unstable substance which released ammonia in the hot extracts was amide since the amide content of the cold extracts (by hydrolysis with 6N HCl for 3 hours suitably corrected for the free ammonia obtained by distillation with magnesia) exceeded that of the hot extracts by an amount equivalent to the extra ammonia the latter contained. VAN SLYKE determinations made on the hot and cold extracts showed, however, that there was also an apparent loss of amino-nitrogen in the hot extracts. The "apparent" amino-nitrogen which decomposed as a result of prolonged, hot (100° C.) extraction at the pH of the tissue extract (5.8 to 6.0) corresponded to 80 to 90 per cent. of the unstable nitrogen present and *did not reappear as ammonia,* since the latter could be ascribed entirely to easily hydrolyzable amide. These results can be explained in the light of the work of VICKERY, CHIBNALL *et al.* (**44**) on the hydrolysis of glutamine. It has been shown that the amide group of glutamine yields ammonia quantitatively (over 98 per cent.) after 2 hours hydrolysis at pH 6 to 7) and moreover that about 80 to 90 per cent. of this amide group also contributes to the "apparent amino nitrogen" which is determined by the VAN SLYKE method. Further, during the hydrolysis of the amide glutamine, there is a concomitant loss of the amino group without the formation of ammonia so that for glutamine the decrease in "apparent amino nitrogen" tended to be double the ammonia released from the amide group. The combined effect of these processes is that the quantitative liberation of ammonia from the amide group of glutamine, which occurs in weakly acid or neutral solutions in two hours, is accompanied by a decrease in the "apparent amino nitrogen" content. VICKERY, CHIBNALL, *et al.* (**44**) emphasize that although a simultaneous loss of "apparent amino nitrogen" accompanying the quantitative hydrolysis of the easily hydrolyzable amide is strong quantitative evidence that the amide concerned is glutamine (or same other substance ca-

TABLE VII

EFFECT OF SALTS AND AERATION ON THE NITROGEN FRACTIONS OF POTATO DISCS. ALL QUANTITIES OF NITROGEN IN MG. PER GRAM OF INITIAL FRESH TISSUE

EXPERIMENTAL TREATMENT OF DISCS	PROTEIN N	SOL. N	AMINO N			AMIDE N			AMMONIA		
			HOT EXT.	COLD EXT.	APPARENT "UNSTABLE"	HOT EXT.	COLD EXT.	EASILY HYDROLYZABLE	HOT EXT.	COLD EXT.	$NH_4 \equiv$ TO UNSTABLE AMIDE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
52.5 hours in aerated 0.00075 M KCl at 23° C.	0.92	1.15	0.90	1.04	0.14	0.129	0.305	0.176	0.178	0.002	0.176
53.0 hours in aerated 0.075 M KCl at 23° C.	1.21	0.85	0.57	0.675	0.105	0.156	0.270	0.114	0.120	0.002	0.118
Original washed discs	0.68	1.38	1.09			0.208	0.348	0.140	0.144	0.002	0.142

pable of forming a pyrrolidone ring) the value of the ratio between these two quantities is too variable to be of use for quantitative measurement. The ratio of the decrease in apparent amino nitrogen to the decrease in easily hydrolyzable amide was observed by VICKERY, CHIBNALL, *et al.* (**44**) to vary from 0.5 to 1.24 for different derivatives of glutamic acid.

Table VIII shows that the "easily hydrolyzable amide" which was de-

TABLE VIII

CONTENT OF UNSTABLE NITROGEN COMPOUNDS IN POTATO DISCS. ALL QUANTITIES OF NITROGEN IN MG. PER GRAM OF INITIAL FRESH WEIGHT

EXPERIMENTAL TREATMENT OF DISCS	APPARENT UNSTABLE AMINO-N	EASILY HYDROLYZABLE AMIDE	NH_3 FROM UNSTABLE N COMPOUNDS	APPARENT UNSTABLE AMINO N / EASILY HYDROLYZABLE AMIDE × 100
	mg.	*mg.*	*mg.*	*mg.*
52.5 hours in aerated 0.00075 M KCl at 23° C.	0.14	0.176	0.176	79.5
53.0 hours in aerated 0.075 M KCl at 23° C.	0.105	0.114	0.118	92.0

composed during alcoholic extraction yielded ammonia quantitatively but that the parallel loss of VAN SLYKE amino nitrogen corresponded to only 80 to 90 per cent. of this and therefore only to the amount reasonably expected from an unstable amide group, such as that contained in glutamine, which also reacts in the VAN SLYKE method. With this reservation and in the light of the statement of CHIBNALL and VICKERY referred to, these data establish a strong presumption that the unstable amide is either glutamine, or a closely related compound. The isolation and complete identification of this unstable amide present in actively metabolizing potato discs will be a necessary part of future investigations. If the "unstable amide" were contained in glutamine, the true amino nitrogen of the hot extracts would be less than that of the cold extracts and the nitrogen concerned in this change would not be estimated in any of the fractions determined. The sum of the amino-nitrogen (of the hot extracts), however, the stable, and the unstable amide, do account for the total soluble nitrogen. It appears, therefore, that the amino groups, if any, of this glutamine-like amide are too stable for this substance to be glutamine itself.

DETERMINATION OF AMIDE AND AMINO NITROGEN IN POTATO DISCS.—Clearly,

therefore, the true measure of the *total amide* content of potato discs is obtained only by the determination of this group by acid hydrolysis at pH 0 for 3 hours on the cold extract of tissue dried *in vacuo* at 60° C. This hydrolysis was appropriately corrected for the free ammonia obtainable by distillation with magnesia. The difference between the figure for total amide and that obtained by hydrolysis at pH 6 for 2 hours (which equals the gain in ammonia content in the hot extracts) is a measure of the easily hydrolyzable amide. The free ammonia content of the discs is obtained from either the cold extract or by direct distillation of the dry powder with magnesia. A measure of the amino nitrogen[7] (free from interference due to the unstable amides) is obtained by the application of the VAN SLYKE method to the hot alcoholic extract. Table IX shows that these fractions

TABLE IX

SOLUBLE NITROGEN OF POTATO DISCS. ALL QUANTITIES IN MG. PER GRAM OF INITIAL FRESH WEIGHT

EXPERIMENTAL TREATMENT OF DISCS	TOTAL SOL. N	AMINO N	TOTAL AMIDE	NH_4	AMINO + AMIDE + NH_4OH NITROGEN
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
52.5 hours in aerated 0.00075 M KCl at 23° C.	1.15	0.90	0.305	0.002	1.207
53.0 hours in aerated 0.075 M KCl at 23° C.	0.85	0.57	0.270	0.002	0.842
Original washed discs	1.38	1.09	0.350	0.002	1.442

account accurately for the total soluble nitrogen of the tissue. Since the amide fraction, which is stable in neutral or weakly acid extracts, is probably asparagine a probable figure may be derived for the "non-asparagine amino nitrogen" by deducting from the total amino nitrogen (determined as above) an amount equivalent to the stable amide fraction. This is probably the best measure available from these data of the amino content of the free amino acids.

SOURCE OF THE NITROGEN INVOLVED IN PROTEIN SYNTHESIS.—Table X compares the changes in the soluble nitrogen and its component fractions with the gain of protein nitrogen per gram of initial fresh weight which occurred in potato discs during experiments in which they were exposed to dilute salt solutions and the standard conditions conducive to salt accumulation and

[7] Excluding the amino group of glutamine, if present, but including that of asparagine.

TABLE X

NITROGEN FRACTIONS INVOLVED IN PROTEIN SYNTHESIS IN POTATO DISCS. ALL AMOUNTS OF NITROGEN IN MG. EQUIVALENTS PER GRAM INITIAL FRESH WEIGHT

EXPERIMENTAL SOLUTION		HOURS	GAIN PROTEIN N	LOSS SOLUBLE N	LOSS OF NH_2-N		LOSS OF NON-ASPARAGINE AMINO NITROGEN		LOSS OF TOTAL AMIDE		LOSS OF STABLE AMIDE (ASPARAGINE?)		GAIN OF UNSTABLE AMIDE		LOSS OF NH_2-N + TOTAL AMIDE	
SALT	CONCENTRATION EQUIVALENTS PER LITER				AMT.	AS PER CENT. OF LOSS OF SOL. N	AMT.	AS PER CENT. OF LOSS OF SOL. N	AMT.	AS PER CENT. OF LOSS OF SOL. N	AMT.	AS PER CENT. OF LOSS OF SOL. N	AMT.	AS PER CENT. OF LOSS OF SOL. N	AMT.	AS PER CENT. OF LOSS OF SOL. N
			mg.	*mg.*	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%	*mg.*	%
$CaCl_2$	0.00075	53.1	0.18	0.18	0.14	78	0.10	55.6	0.008	4.5	0.041	22.8	0.033	18.3	0.15	83
KCl	0.00075	52.5	0.24	0.23	0.19	83	0.12	52.3	0.029	12.6	0.074	32.1	0.045	19.5	0.22	96
KCl	0.075	53.0	0.55	0.53	0.52	98	0.47	90.6	0.078	14.7	0.052	9.8	−0.03	−5.6	0.60	112
$CaBr_2$	0.00075	88.0	0.35	0.39	0.36	92	0.21	53.9	0.082	21.0	0.15	38.5	0.068	17.4	0.44	113
KBr	0.00075	63.5	0.28	0.29	0.31	107	0.21	72.5	0.021	7.3	0.10	34.5	0.079	27.2	0.33	114
KBr	0.075	63.0	0.65	0.61	0.56	92	0.45	73.7	0.118	19.4	0.11	18.0	−0.08	13.1	0.68	111

high respiration. The agreement between the gain of protein nitrogen and the decrease of soluble nitrogen is close. The table shows (column 6) that almost the entire amount of the soluble nitrogen used in protein synthesis was derived from the amino nitrogen fraction and when the maximum[8] allowance is made for that part of the amino nitrogen which might have been derived from the asparagine metabolized (column 7) it is clear that amino compounds other than asparagine, which in the light of the titration curve data must be amino acids, contribute most of the nitrogen used in the synthesis of protein by the potato cells. The table also shows certain specific effects of the salts. The stronger potassium chloride solution increased the protein synthesis. This confirms a result which has already been noted (table VII). The table, however, shows that the effect of the salt (KCl) on protein synthesis could have been entirely at the expense of the reserve of non-asparagine amino (amino acid) nitrogen. In the presence of dilute calcium solutions the non-asparagine amino nitrogen contributed much less of the total used in protein synthesis than when the tissue was in contact with the stronger aerated potassium salts (column 7).

The conclusion, therefore, is that the nitrogen involved in protein synthesis is drawn principally from amino compounds (amino acids) other than asparagine or glutamine but also that the salts present affect not only the total amount of protein synthesized but also the source from which the nitrogen is obtained. Increased concentrations of potassium salts, which increase the total synthesis, also increase the relative utilization of the amino acids whereas the calcium salts, which depress the total synthesis, also decrease the utilization of the amino acids relative to other compounds.

The status of the amide fraction of the soluble nitrogen is complicated by the formation during the period of active metabolism of easily hydrolyzable amides. Protein synthesis (table X) was also accompanied by a decrease in the total amide fraction and this, together with the observed loss of amino nitrogen, accounts for the entire change in soluble nitrogen with an error which is probably not excessive in view of the complications in these determinations. The change in the total amide, however, conceals a much greater utilization of the more stable amides (asparagine) which is offset by the accumulation, in the actively metabolizing tissue, of easily hydrolyzable amides. In the light of the evident connection between respiration and nitrogen metabolism, the extreme reactivity of this substance, its apparent increase when the conditions of oxygen and temperature permit rapid metabolism (*e.g.* in dilute salt solutions), and its further depletion when, in strong potassium salts, protein synthesis is stimulated, are all features which might suggest that this substance may be a reactive intermediary between the stable reserves of amino acid amide on the one hand and protein on the

[8] If any of the disappearance of "stable amides" is accounted for by the conversion of asparagine to aspartic acid, which remains as such, this correction is too great.

other. These considerations arouse an interest in the nature and reactions of this glutamine-like component of the soluble nitrogen fraction which is not overshadowed by the greater abundance in the tissue of the more stable nitrogenous substances.

Titration curves of potato sap

The determination of titration curves on the expressed sap of plants reveals the effect of experimental treatments on the constituents of the buffer complex. Since the components of the buffer system include certain substances which are not readily determined quantitatively (*e.g.* amino acids and organic acids) this determination has considerable value. For this purpose, however, the buffering in the region of the pH of the sap—with which most investigations have been concerned—is not especially instructive and the titration must be extended far beyond this to reactions both more alkaline and more acid. See Small (**32**, pp. 265–291) for a discussion of the buffering of potato sap between the limits pH 4.5 to 7.0. Dunne (**8**) has utilized this method of approach in connection with problems of mineral nutrition, and Hoagland and his collaborators have freely utilized it as an indirect means of investigating the chemical changes which accompany the uptake of salt in barley roots. The authors were familiar with the latter investigations and hence applied this technique in the case of potato discs.

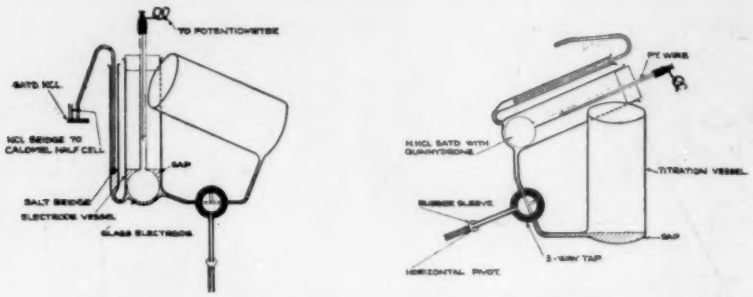

Fig. 4. Rocking electrode and titration vessel for the determination of titration curves of sap.

The titration curves were carried out on aliquots of potato sap freshly expressed from discs which had been frozen and stored at – 20° C. after the conclusion of the experimental treatment they received. The change of pH with the addition of 0.20 N HCl, between the pH of the sap and about pH 2.5, was recorded; for the alkaline range to pH 11.5 the treatment was repeated on another aliquot of the sap using 0.20 N NaOH. The amount of acid or alkali added was arbitrarily adjusted according to the shift in pH obtained in the region concerned. The earlier curves (figs. 6 and 7) were

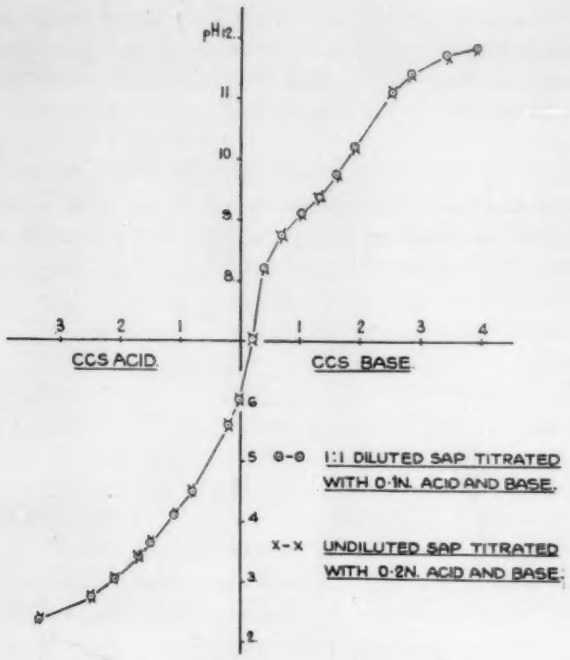

FIG. 5. Titration curve of potato sap.

made from readings obtained using a hydrogen electrode by A. ULRICH[9] although subsequent determinations made with a glass electrode show that the type of curve obtained is not dependent upon a particular method of recording the E.M.F.

The titration curve of potato sap can be more rapidly and effectively explored by the use of a glass electrode system in conjunction with a potentiometer of the Electrometer Valve pattern of the Cambridge (England) Instrument Co. This instrument permits readings to be obtained which are automatically corrected for temperature and which can be stated in E.M.F. or pH units with an accuracy which corresponds to 0.01 pH unit. A convenient form of combined electrode and titration vessel is that described in figure 4 A and B. The necessary additions of acid or alkali are made (from burettes reading to 0.01 ml.) to the sap sample which is contained in the titration vessel, placed for the purpose in the position of figure 4 A. In the position shown in figure 4 B the liquid undergoing titration flows by gravity into the glass electrode chamber and covers the bulb of the electrode. The combined electrode and titration vessel rocks from the position of figure 4A to that of figure 4B on an axis provided by a horizontal portion of the outlet tube. Repeated passage of the liquid from the electrode chamber to the

[9] Division of Plant Nutrition, Berkeley, Cal.

titration vessel insures efficient mixing. The glass electrode bulb contains N HCl, saturated with quinhydrone into which dips a platinum wire. The electrical connection between the glass electrode vessel and the calomel half cell is made (in the position of fig. 4B) by a salt bridge and a saturated potassium chloride solution.

The combined rocking electrode and titration vessel mounted on a hard wood base was so designed that it worked with a minimum of 4 ml. of liquid. To economize material, 2 ml. of sap plus 2 ml. of water were used for each of the titrations with acid and base. It was verified experimentally that dilu-

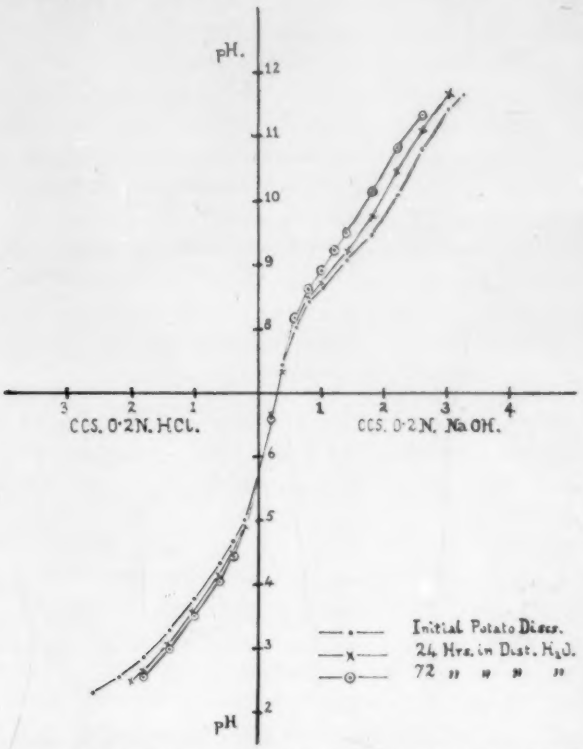

FIG. 6. Changes in the buffer system of potato discs during metabolism at 23° C. in aerated distilled water—the effect of time.

tion in the ratio of 1:1 did not change the nature of the buffer system of the sap. This is evident from figure 5 which shows the titration curves of undiluted potato sap using 0.2 N acid base and also of sap diluted 1:1 with 0.1 N acid and base.

EFFECT OF SALTS AND METABOLISM ON THE BUFFER SYSTEM OF POTATO

The effect on the titration curve of potato sap of the metabolic processes which occur in the discs in distilled water, and under the conditions of

temperature and aeration conducive to maximum respiration and salt accumulation was determined. In figure 6 can be seen the titration curves of the sap which was expressed after freezing from the initial discs, prior to their period of intense metabolism, and also after periods of 24 and 72 hours, respectively, of high respiration.

Normally the sap of the potato is most strongly buffered between pH 8.5 to 9.5 (figs. 5 and 6), the region in which the buffer effect of amino acids is at its maximum.[10] Progressively with time the metabolic processes in the

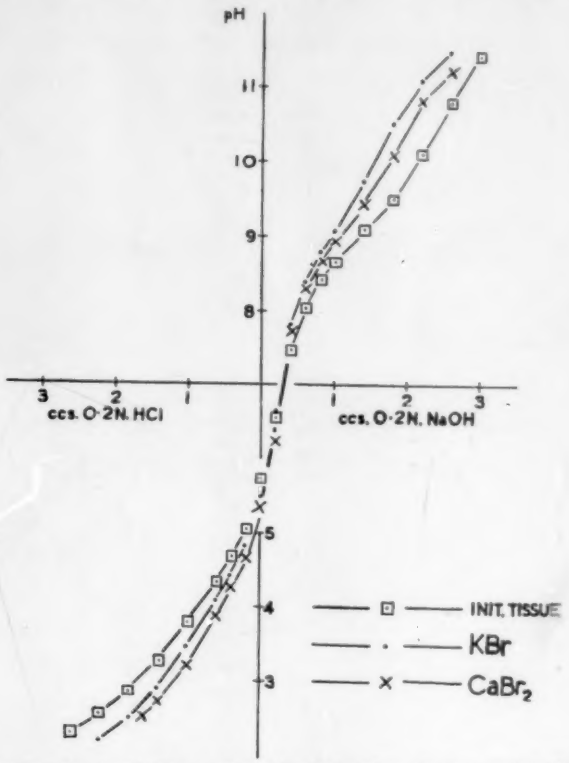

FIG. 7. Changes in the buffer system of potato discs during metabolism at 23° C. in aerated solutions—the effect of salts (KBr, $CaBr_2$).

thin, aerated discs in water cause a decrease in these buffer components which is to be correlated with their conversion to substances with little buffer value, *e.g.*, protein. As figure 7 shows the decrease of constituents which buffer between pH 8 and 10 is greater when the tissue is in contact at 23° C. with aerated solutions of potassium salts than calcium salts—a result which is in agreement with the finding that the former accentuate and the latter

[10] The pKa values for some typical amino acids are as follows: glycine 9.7, aspartic acid 9.9, glutamic acid 9.8, tyrosine 9.4, phenylalanine 8.6.

depress protein synthesis from the stored amino compounds of the potato tissue.

Since the changes which occur in that portion of the titration curve which lies between the limits pH 8.0 to 10 are closely related to the metabolism of the cells, it is desirable to specify the substances involved. Further proof that amino acids are responsible is provided by the data in figure 8,

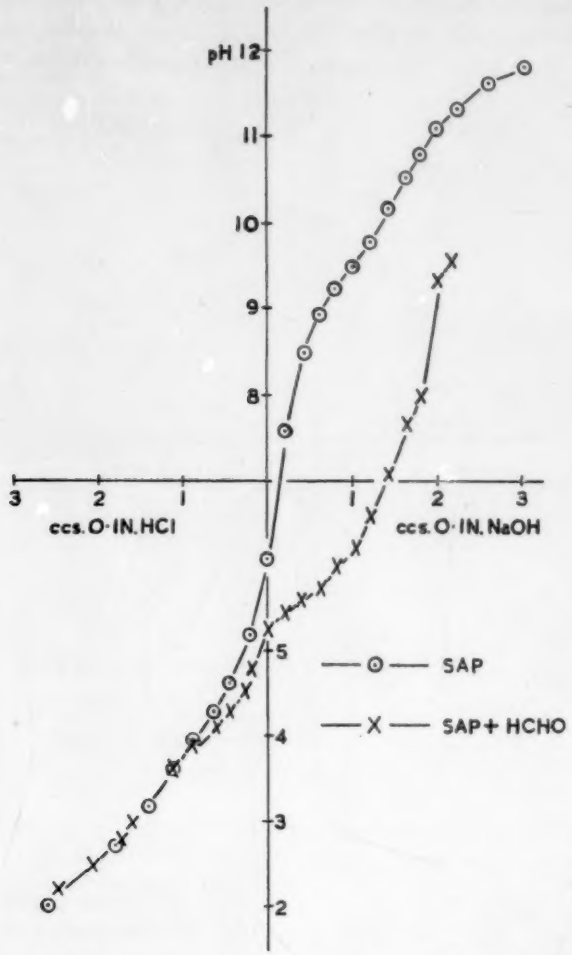

FIG. 8. Effect of formaldehyde on the titration curve of potato sap.

which shows the titration curve of diluted potato sap (2 ml. sap plus 2 ml. H_2O) with and without the addition of neutralized formaldehyde (4 ml. of 44 per cent.). In the acid reactions (more acid than pH 4.0) the two curves coincide, but in presence of formaldehyde the buffer capacity normally found

in the region pH 8 to 10 is displaced to the region pH 5.0 to 6.5—a region in which the sap is normally only weakly buffered. The compounds involved must therefore be amino acids which in presence of formaldehyde form methylene-imino derivatives which, being stronger acids, have their maximum buffer effect at a lower pH (pKa values of the methylene-imino derivatives of common amino acids range from 5.4 to 6.9).

It still remains to determine whether the amino-acid moiety (pKa = 8.87) of the asparagine present in potato tissue could account for the changes which occur in the buffer value in the region of pH 8 to 10 during metabolism. SMALL (30) estimated that asparagine contributed only 5 per cent. of the buffer value of potato sap in the region pH 6.7. Figure 9, which

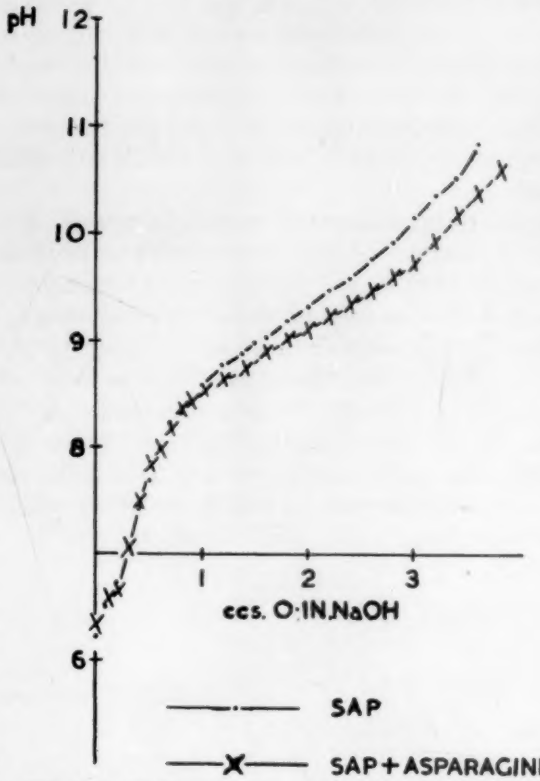

FIG. 9. Effect of added asparagine on the titration curve of potato sap.

shows the titration curve of 2 ml. of potato sap, with and without the addition of an amount of asparagine such that its amino-N content was equal to the total soluble nitrogen of the normal sap. It is evident that *per unit of soluble nitrogen* the normal sap has much greater buffer value than the amino-acid moiety of the asparagine which it contains.

The conclusion, therefore, is that the substances which buffer the normal sap strongly in the region of pH 8 to 10 are amino acids other than asparagine and that the changes induced in this part of the titration curve due to the combined effects of alkali halides and metabolism reflect the effect of these treatments upon the amino acid content of the tissues. This conclusion is in agreement with that obtained by the direct determination of the components of the soluble nitrogen.

At reactions more acid than the natural sap there is a considerable degree of buffering which must be attributed to organic acids. According to SMALL (**30**) citrates and phosphates might contribute 60 per cent. of the buffering between pH 6 and 7 where, however, the buffering is weak; but at reactions more acid, their contribution is much less *e.g.*, 19 per cent. at pH 4.5. SMALL was led to the conclusion that even at reactions as near neutral as pH 5.0 other organic acids were involved and he demonstrated the presence of ether soluble organic acids. From the curves given in figures 5, 6, and 7 it seems clear that the strongly buffered sap at a pH more acid than 5.0 must owe this property to organic acids and their identification is an imperative task for the future.

The progressive metabolism which occurs in aerated distilled water is accompanied by a continual decrease in the buffer value of the sap at reactions between pH 2.0 and 6.0, and this must mean that organic acids are being consumed. An effect of salts on the organic metabolism of the potato discs is suggested by the greater decrease of the buffer value between pH 2 and 6.0, which occurs when the tissue is exposed to aerated calcium bromide, than when it is respiring in aerated potassium bromide. This is an example of a phenomenon observed frequently by HOAGLAND[11] and associates on barley roots. Both potato and barley results are consistent with the view that the bromide ion is absorbed from calcium bromide solutions mainly unaccompanied by the cation so that organic anions disappear from the sap and bicar-

TABLE XI

EFFECT OF DRYING ON ASCORBIC ACID CONTENT OF POTATO DISCS

SERIES	FRESH WEIGHT OF SAMPLE	STATE OF TISSUE WHEN EXTRACTED	ASCORBIC ACID PER GRAM FRESH WEIGHT
	gm.		*mg.*
I	7.50	Fresh + NaCN	0.164
	7.80	Dry	0.188
II	7.50	Fresh	0.170
	7.50	Dry	0.172

[11] Results awaiting publication by ULRICH and by HOAGLAND and BROYER privately communicated to the authors.

bonate ions accumulate in the external solution (**36**) which also becomes more alkaline. The titration curves, therefore, not only extend and confirm the conclusions on the nature of the nitrogen metabolism which were obtained from direct determinations but they also suggest that the potato cells metabolize organic acids in amounts which are determined by time and the nature of the salt present. With reference to the rôle of organic acids, the conclusions from the titration curves stand alone, as direct analyses of organic acids have not been made. Clearly the above account does not distinguish between the buffer effect attributable to nitrogen-free organic acids and to organic acid radicles contained in polybasic amino acids.

Behavior of potato oxidase, ascorbic acid, and glutathione in the actively metabolizing discs

Under the conditions conducive to salt accumulation potato discs turn slightly brown owing to the activity of the familiar oxidase system of the potato cells which acts upon phenolic compounds (including tyrosine) stored therein. This behavior, noted earlier (**34, 36, 39**) is sensitive to the oxygen pressures which also affect respiration. It is, therefore, a result of no little interest that the effects induced by salts upon respiration are paralleled by visible effects on the oxidation of phenolic compounds—potassium salts increase and calcium salts decrease the browning reaction. The specific effects of the salt are also increased with concentration. It is clear that the cellular oxidation of phenols and respiratory substrates is similarly effected by salts and oxygen even if the same catalytic mechanism is not involved.

The effect of neutral salts, other than those which contribute to buffer solutions, on the activity of the aerobic oxidase preparations isolated by Raper and his colleagues from potato tissue does not appear to have been studied in detail. Samisch (**28**) made an intensive investigation of the effect of salts on the oxidase which causes darkening of fruits (apricots). However, the experiments of Samisch on the extracted enzyme reveal no sign of a contrasted effect of potassium and calcium salts such as the organized living cells of potato reveal. In his work, on the contrary, increased concentrations of alkali (*e.g.*, Na) halides *decreased* the activity of oxidase extracts in presence of catechol.

Szent Gyorgyi (**39, 40**) showed that the substance now known as ascorbic acid could reduce the oxidized products produced by potato oxidase from those substances which contain the catechol group, and believed that ascorbic acid could provide for the reversibility of this catalytic system. The first products (quinones) of the oxidation of the phenolic substances of potato are reduced to the phenolic condition by ascorbic acid, which is in turn oxidized. Johnson and Zilva (**13**) found that the enzyme (phenolase) does not act upon ascorbic acid directly but that this substance is oxidized by those inter-

mediates (orthoquinones) which also deaminate amino acids. From the work of KEILIN and MANN (14) on purified polyphenol oxidase, which is apparently a copper-protein compound, it is also clear that the oxidase oxidizes ascorbic acid only in the presence of catechol.

Accordingly attention was turned to the reducing substances to be found in cells and particularly to two substances (ascorbic acid and glutathione) both of which are commonly supposed to bear a close relation to metabolic activity, to be capable of reversible oxidation and reduction, and which have been freely incorporated into the proposed catalytic systems of cells. The expectation was that increased amounts of the brown, oxidized products might be attributed to, or accompanied by, a decreased concentration of the reduced form of the substances in question. It can be stated at once that no clear relationship of this kind between the browning effects and the behavior of either ascorbic acid or glutathione in the living cells has yet emerged. The observations made, however, present yet another feature of the metabolism of potato discs which is of particular interest since the substances involved are supposed to be connected with, if they are not causal agents for, vital activity.

THE DETERMINATION OF ASCORBIC ACID IN POTATO DISCS

The familiar 2:6 dichlorophenolindophenol titration was applied. The dye was used in solutions of 0.10 gm. in 200 ml. of solution buffered at pH 6.8 with a phosphate buffer. The TILLMANN reagent was standardized by a solution freshly prepared from lemon juice assuming that all of the reducing value (by iodine) of this extract was due to ascorbic acid.

As others have found, the difficulty is to standardize the extraction technique. Discrepancies between different workers and methods have led to much controversy in the literature which cannot be dealt with here. Only a brief statement of the method applied and the results obtained will be attempted.

If potato tissue is extracted for ascorbic acid as directed by BESSEY and KING (5) in 8 per cent. acetic acid, 90 per cent. of the initial activity is lost in a period as short as 20 minutes. Fresh tissue, however, ground in 8 per cent. acetic acid + M/10 NaCN gave a constant ascorbic acid titre during a period of two hours after the extraction and, after standing overnight exposed to air, 84 per cent. of this original activity still remained. If the extraction technique was preceded by vacuum drying for $\frac{3}{4}$ hours at 100° C. slightly higher (up to 20 per cent. greater) values were obtained than when extraction took place from the fresh tissue in presence of cyanid (table XI). Some (19) in this sort of difference evidence for combined and uncombined ascorbic acid whereas others (18, 38) regard these differences as caused by the more effective inactivation of enzymes in the heated tissue. Since such small differences have not yet been correlated with metabolism, only that method

which gave the best determination of the reduced ascorbic acid was applied (extraction of tissue, vacuum dried, in presence of cyanide). The ascorbic acid exists in washed, living discs *only* in the reduced form. If extracts were made as above, saturated with hydrogen sulphide, and the latter removed in a vacuum in the presence of cyanide, the titrations were the same as for the extract untreated with hydrogen sulphide.

Effect of time on the ascorbic acid content of potato discs in aerated salt solution

In thin potato discs in aerated solutions at 23° C. a progressive *increase* in the content of ascorbic acid occurs (see two series of table XII). This

TABLE XII

Ascorbic acid content of potato discs in aerated salt solutions at 23° C.

Series	Hours	Ascorbic acid per gram fresh weight
		mg.
KCl (0.001 equivalents, per liter)	0.0	0.094
	75.5	0.161
	125.0	0.198
$CaCl_2$ (0.001 equivalents, per liter)	0.0	0.094
	75.5	0.155
	125.0	0.188

occurs in tissue in contact with aerated distilled water at 23° C. and is yet another symptom (there is no reason to believe that it is a cause) of the increased metabolism which, although it can proceed independently of salt absorption, is a prominent feature of the system which is capable of absorp-

TABLE XIII

Effect of aerated salt solutions and time on the content of reduced ascorbic acid and glutathione in potato discs

Composition	External solution. Salt concentration per liter	Sample	Period hours	Ascorbic acid per gram initial fresh weight	Glutathione per gram initial fresh weight
				mg.	*mg.*
.....	.....	Initial	0.0	0.104	0.064
.....	.....	Initial	0.0	0.102	0.059
H_2O	.....	Final	70.0	0.151	0.059
KCl solution	0.05	Final	70.0	0.163	0.056
$CaCl_2$ solution	0.05	Final	70.0	0.162	0.060
H_2O	.....	Final	115.5	0.189	0.071
KCl solution	0.05	Final	115.5	0.199	0.067
$CaCl_2$ solution	0.05	Final	115.5	0.213	0.065

tion. The salts which do affect respiration (KCl which increases it, and $CaCl_2$ which decreases it) have only a little, and barely significant effect on the final, total ascorbic acid content (compare the data for the KCl and $CaCl_2$ series at the same time periods in tables XII and XIII). If—against the weight of evidence—the total ascorbic acid content does include a component which is either combined or in intimate association with the protoplasm it is still possible that the salt treatments affect the combined fraction more significantly than these determinations of total ascorbic acid content indicate.

Effect of time and salt solutions on reduced ascorbic acid and glutathione in potato discs

The simultaneous effects of the salt and aeration treatment upon the glutathione and ascorbic acid content of potato discs was examined (table XIII). Glutathione, to which a special rôle in biological oxidation has been ascribed, is now known to bear a definite relationship to the oxidation of ascorbic acid by the so-called "hexo-oxidase" of Szent-Gyorgyi (**41**). Hexo-oxidase is a specific enzyme of the expressed juice of cabbage, which Zilva (**45**) thought was present in apple and which oxidizes ascorbic acid (hexuronic acid) to dehydro-ascorbic acid. According to Hopkins and Morgan (**12**) the enzyme hexo-oxidase does not oxidise glutathione directly, but in mixtures of glutathione and ascorbic acid the former is oxidized at the rate at which the latter would have disappeared if the glutathione had been absent. In other words the ascorbic acid is oxidized and the glutathione promptly reduces it, so that ascorbic acid behaves like a co-enzyme in the oxidation of glutathione by "hexo-oxidase" and itself remains in the reduced condition.

After the treatments indicated in table XIII the potato discs were vacuum dried and extracted with acetic acid. In the one case (determination of ascorbic acid) the extract was titrated when standardized ascorbic acid reagent (2 : 6 dichlorophenolindophenol) and in the other, for the determination of total reducing value (ascorbic acid + glutathione), with 0.05 N iodine according to the method of Hopkins and Morgan (**12**).

The data (table XIII) show again that, at 23° C., in thin discs in aerated solutions, the content of ascorbic acid rises in a manner which bears an approximately linear relationship to time. Comparison of tables XII and XIII with figure 3 verifies that the change in the ascorbic acid content of the discs with time does not follow the same course as the respiratory time drift, (*e.g.*, tissue in distilled water) neither is there an outstanding effect which may be attributed to the presence of salts. Glutathione (reduced) contributes only a small part of the total reducing value and the quantity present in the discs does not change significantly when they are exposed to aerated salt solutions.

These data show that the total ascorbic acid content of the living cells is not the factor which directly determines either the browning reaction referred to or the respiratory rate of the cells. The salt effects upon respiration, protein synthesis, and oxidase action in the living cells must either be localized in centers distinct from the milieu in which the ascorbic acid occurs or else the ascorbic acid is protected against oxidation by other unknown substances. The progressive browning of discs with time is not compatible with the continued increase of their reduced ascorbic acid content and, neither in its relation to time nor to salts is the ascorbic acid content of living potato discs affected in a manner similar to respiration. Future work must determine the nature of the substance, or circumstances, which prevents the ascorbic acid in the living cells from being oxidized by the enzyme present. The now familiar rôle of ascorbic acid as a potential carrier of oxidation as, *e.g.*, between hexo-oxidase and glutathione, suggests that it may enable phenolase to activate a much wider range of substances than its appraised specificity would indicate. Hence, although the quantity of ascorbic acid cannot as yet be correlated with the respiration rate, or even the browning reaction, its presence in actively metabolizing potato discs may yet prove to be essential to the processes they carry out.

A possible relationship between the sugar and ascorbic acid content of potato discs is suggested by the fact that the increase of ascorbic acid occurs during a period of starch hydrolysis, and this requires further investigation. Such relationships, however, like the recognition that ascorbic acid is more concentrated in cells which show vital activity, *e.g.*, the apical regions of roots (**26**), or the young shoots on resting potato tubers (**22**), does not yet justify the supposed regulatory rôle of this substance in the metabolic processes which involve oxidation since it does not distinguish between ascorbic acid as a product and as a primary cause of the vital activity in question.

One can only repeat that the facts are that salts (of K and Ca) do influence the metabolism of potato discs. They exert an effect in the cells upon the activity of the enzyme system which catalyzes the oxidation of phenolic compounds and *in vitro* produces oxidative deamination of amino acids; the suggestion is that the latter processes provide the link with the rate of aerobic respiration and protein synthesis. As yet, however, similar effects of salts have not been demonstrated *in vitro* in preparations which lack the organization of the living cells; and therefore the mechanism of the salt and oxygen effects described cannot be ascribed solely to the oxidase system of the cells. It also involves the more intimate part of the living system which, in conjunction with the system which catalyzes oxidation, brings about protein synthesis. A large number of experiments have been made to investigate the effect of potassium and calcium salts on the browning of tissue extracts, on the oxidation of catechol and of ascorbic acid by crude

potato enzyme preparations, and on the autoxidation (catalyzed by copper) of ascorbic acid. It is clear from these that the work must be continued with purified enzyme preparations.

Summary

The theoretical implications of the data have been elaborated in the text. It remains only to summarize and recapitulate the essential results and conclusions.

The variability in the composition of replicate batches of 40 to 60 standard potato discs, cut from a uniform stock of tubers and washed for 24 hours in running tap water, is small and negligible relative to the changes which occur due to metabolism during periods of the order of 72 hours under the conditions conducive to salt absorption (40 to 60 discs in 2 liters of aerated solution at 23° C.).

Since the fresh and dry weight of the discs changes during the course of metabolism the best basis for the calculation of the results is the initial fresh weight of a known number (40 or 60) of standard discs of known weight (30 or 45 gm.).

The application of conductivity methods to the determination of absorbed carbon dioxide is described. By this means the effect of salts on the time drift in the respiration of potato discs has been investigated.

In relatively strong (0.05 equivalent per liter) potassium salt (KCl) the tissue approaches a steady respiratory rate at a level which far exceeds that of tissue in distilled water and, conversely, in a calcium solution ($CaCl_2$) of the same equivalent strength the steady level (attained after 30 hours) is much lower than that for distilled water. The contrast in the effect of potassium and calcium salts is one which reappears in many aspects of the metabolic behavior of the tissue.

The direct measurement of the heat evolved by potato discs during their metabolism under the required conditions is beset with technical difficulties. The over-all change in the caloric value of the discs, as a result of their metabolism, can be measured by the use of standard bomb calorimetry. The discs decrease in calorific value by amounts much in excess of expectations on the basis of their respiration.

A scheme for the analysis of potato discs has been described. This reveals the changes which occur in the dry weight, fresh weight, starch, sugar, soluble nitrogen and protein content of the discs. Two independent methods show that the estimate of protein synthesized is not appreciably affected by the peculiarities of a single method.

Discs of potato in aerated distilled water or potassium salt solutions synthesize protein. The synthesis is greater in relatively strong potassium salts (KCl) than in water and it can be suppressed in relatively strong (0.075 equivalents per liter) calcium solutions ($CaCl_2$ or $CaBr_2$). The nitrogen for synthesis is drawn from the soluble nitrogen fraction.

The soluble nitrogen fraction of dormant potato tubers consists of both amides and amino compounds and only negligible amounts of free ammonia. The total amide contains two components: the one, probably asparagine, which is stable under hot alcoholic extraction and only hydrolyzed by 6N HCl under reflux condenser for 3 hours and the other, similar to glutamine, which is unstable under hot alcoholic extraction and is hydrolyzed by 2 hours at pH 6 to 7. The accompanying changes in the VAN SLYKE amino nitrogen fraction show that the easily hydrolyzable amide groups behave like that in glutamine. Recognizing these complications, methods are described which permit the determination of the stable amide, the "heat unstable amide" and the amino-nitrogen fraction (free from confusion with the amide group of glutamine-like substances). The fractions determined account quantitatively for the soluble nitrogen content of the tissue.

During protein synthesis nitrogen is drawn from both the stable amide fraction and the amino nitrogen fraction. The bulk of the nitrogen converted to protein is derived from amino compounds other than asparagine. Salts (KCl and $CaCl_2$) influence both the total synthesis of protein and the relative utilization of amino acids and other compounds. Potassium salts increase, and calcium salts decrease, the relative utilization of the amino acids.

Under the conditions of active metabolism and protein synthesis the unstable, glutamine-like amide, increases in the tissue. It appears to be a reactive intermediary and its subsequent utilization is accelerated by the salt conditions which stimulate synthesis.

The metabolic processes described above are reflected in changes in the buffer system of potato. Methods suitable for the rapid electrometric titration of potato sap are described. Titration curves show a strong buffering (pH 8.0–10.0) which cannot be attributed to asparagine; it is shown to be due to amino acids. The amino acid buffering decreases as synthesis of protein occurs. The salts which affect synthesis also affect the titration curve of the sap. Simultaneous effects upon that portion of the titration curve in which organic acid radicals are effective suggest that these also diappear during metabolism in distilled water and their utilization is still greater in calcium bromide solutions.

The salt and oxygen treatments (aerated KCl solutions) which stimulate the respiration of potato discs also increase the superficial browning which is due to the oxidation of phenolic compounds by the aerobic oxidase of potato. The converse effect is observed in aerated calcium chloride solutions. It is believed that the parallel effects of salts on the activity of the oxidase and protein synthesis are to be ascribed to the deaminating action of substances (ortho-quinones) which are intermediates in the oxidation of the phenolic compounds.

The bulk of the reducing (by iodine) action of potato extracts is due to

ascorbic acid. Special precautions are described which nullify the effect of the tissue oxidase on the ascorbic acid in extracts and the determination of the reduced form of ascorbic acid in the potato discs is described. During the rapid metabolism which occurs in tissue in aerated distilled water the reduced ascorbic acid content of potato discs increases progressively with time. Neither in its relation to time, nor to salts, does the ascorbic acid content of potato discs appear to be a causal agent in the respiration or the browning reaction of the living cells. The content of reduced glutathione in the tissue is not significantly affected by the conditions conducive to salt absorption or by the nature of the salt supplied. The possible rôle of ascorbic acid as a carrier of oxygen between the phenolase and non-phenolic substrates is recognized.

The outstanding metabolic processes of potato discs under the conditions conducive to salt accumulation are those which are conditioned by oxygen and the nature of the salts supplied and these are mutually inter-related. Of these the synthesis of protein, the utilization of amino-acids and stable amides, the formation of unstable amides, the use of organic acid radicals and the oxidation phenomenon shown by the browning reaction in the living cells, are all linked with the rate of aerobic respiration and must contribute to the metabolic machinery which renders salt accumulation possible. Only future work can tell which, if any, of these diverse processes is more intimately concerned than all the rest.

This paper is based on work which has been in progress since 1933 and which, started in the Division of Plant Nutrition, University of California, has been continued in the Department of Botany, Birkbeck College, University of London. Collaboration with C. PRESTON was confined to the work at Birkbeck College. Our grateful appreciation of the support which the work received from both laboratories is here acknowledged, and our thanks are accorded to Prof. HOAGLAND for reading the proofs. A grant from the publication fund of Birkbeck College assisted the preparation and publication of figures.

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BIRKBECK COLLEGE
UNIVERSITY OF LONDON

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ROOT RESISTANCE AS A CAUSE OF DECREASED WATER ABSORPTION BY PLANTS AT LOW TEMPERATURES

PAUL J. KRAMER

(WITH FIVE FIGURES)

Introduction

It is well known that low soil temperatures decrease the absorption of water by plants. Many years ago SACHS (**24**) reported that tobacco and gourd plants growing in moist soil under conditions favoring a low rate of transpiration wilted when the soil was cooled to 3° to 5° C., but recovered when the soil was warmed to 12° to 18° C. Cabbage and turnips were less affected, absorbing enough water at a soil temperature just above freezing to prevent wilting during times of moderate transpiration. A few years later VESQUE (**29**) made further studies using a potometer method in which the roots of *Hedera helix* were immersed in water and cooled to low temperatures. He reported that absorption by transpiring plants decreased as the temperature was decreased although some absorption occurred even at 0.5° C. The most rapid decrease occurred between 15° and 10° C. VESQUE stated that since temperatures higher than 15° C. sometimes resulted in an increase and sometimes in a decrease in absorption, it was impossible to decide as to the real effect.

KOSAROFF (**17**), by the use of potometers, made an extensive investigation of the effects of low temperature on water intake through living root systems and root systems killed by scalding. He found that lowering the temperature from about 20° C. to freezing retarded the absorption of water by living root systems of Phaseolus and Pisum 25 or 30 per cent. but did not decrease the absorption of water through dead root systems. Lowered temperature decreased water intake through cut branches of woody plants, but not as much as through living root systems. KOSAROFF also reported that certain species including *Sinapis alba* and *Chrysanthemum indicum* were able to absorb water from soil at −1° C.; *Chrysanthemum indicum* and Salix absorbed water from ice in which their roots were frozen. Since the claim that absorption occurred was based on recovery of wilted leaves in a humid atmosphere, it might be supposed that recovery was brought about by movement of water from stem to leaves rather than by absorption through the roots. KOSAROFF discounted this explanation, however, since shoots not in water or ice failed to recover their turgor in a saturated atmosphere.

STAHL (**25**) reported guttation from the leaves of oats, barley, wheat, and *Geranium pyrenaicum* with roots in soil cooled to approximately 0° C. This, he believed, indicated that some absorption was occurring even at freezing. DUNCAN and COOKE (**12**) found that the rate of absorption by sugar cane

plants decreased as the water in which their roots were immersed was cooled from 28° to 10° C. They also state that sugar cane plants growing in well watered soil have been observed to wilt upon cooling the soil to about 8° C.

CLEMENTS and MARTIN (7) investigated the effects of soil temperature on the rate of transpiration of *Helianthus annuus*. The rate of transpiration decreased but slightly with decreasing soil temperature from 37° to about 13° C., but decreased rapidly below 13° C., being reduced to one half at about 3° C. The plants begin to wilt at about 4.5° C. and were completely wilted at 1° C., but rapidly recovered when the soil was again warmed. All plants in these experiments were exposed to similar atmospheric conditions.

ARNDT (1) reported that cotton plants exposed to the sun in an unshaded greenhouse wilted at soil temperatures of 17° to 20° C. and plants in solution cultures wilted at 10° to 18° C.

It is probable that, aside from deficient soil moisture, low soil temperature is the most important environmental factor affecting the rate of water absorption. Considerable so-called winter injury, particularly of evergreens, is really injury from desiccation brought about by bright sun and wind which causes excessive transpiration at times when the soil is frozen, or near freezing, so that absorption is too slow to replace the water lost. MICHAELIS (21) has suggested that this is an important factor in determining the tree line in mountains. WHITFIELD (31) and CLEMENTS and MARTIN (7) also believe that the low soil temperatures occurring at high altitude influence plant growth.

TRANSEAU (28) believed that the xeromorphic characteristics of northern bog plants were caused by slow absorption of water resulting from the low temperatures and poor aeration existing in such bogs. Similar anatomical characters could be produced experimentally by growing plants with low soil temperatures, poor aeration, or in dry sandy soil. FIRBAS (13), on the other hand, reported that low temperatures did not interfere with the absorption of water by plants native to German bogs and decided that decreased absorption resulting from low temperatures could not be a cause of their xeric structure. DÖRING (11) studied the effects on absorption of transferring plants in potometers from 20° to 0° C. and found that while the rate was decreased 70 or 80 per cent. in some species it was not decreased at all in other species.

It is not surprising that soil temperature often exerts a marked influence on the rate of water intake since it can affect the process in several different, although more or less related, ways. The more important of the suggested causes for decreased absorption at low temperatures are as follows:

1. Lowering the soil temperature decreases the rate of movement of water from the soil to the absorbing surfaces of the roots. This effect has not been measured directly with root systems, but the writer (19) found the water-supplying capacity of the soil as measured with soil-point cones to be only

one-half to one-third as great at 0° as at 30° C. WILSON (**32**) also found that the water-supplying capacity was decreased at low soil temperatures. This of course would not be a factor in potometer experiments where the roots are in water. CLEMENTS and MARTIN (**7**) believed it was of little importance in their experiments on plants rooted in soil, but it seems probable that under certain conditions it may be of some importance.

2. Low temperatures retard the elongation of roots. Since the continual extension of root tips into contact with the water films surrounding hitherto untouched soil particles is very important in making the soil moisture available, a decrease or cessation of root elongation will probably decrease the rate of absorption. This would be most important in soils with a moisture content below the field capacity, a condition which commonly exists in the field.

3. The permeability of cells decreases as the temperature is lowered. DELF (**9**) found that the rate of plasmolysis and hence permeability of the cell membranes of dandelion scapes and onion leaves decreased with decreasing temperatures from 35° to 5° C. A temperature above 35° seemed to have an injurious effect on permeability as the tissue shrank even in water above this temperature. STILES and JØRGENSEN (**27**) studied the rate of absorption of water by carrot and potato tissue and found that it decreased with lowering of temperature from 30° to 10° C. According to STILES (**26**) who has summarized the literature on this subject, permeability to water increases with increasing temperature to at least 30° C., above which the time factor appears. The temperature coefficient for the rate of diffusion through cell membranes was found to be much higher than that for diffusion in aqueous solutions. The causes of the apparent decrease in permeability are at least partly considered in the next two paragraphs.

4. The viscosity of protoplasm and of the colloidal gels in the cell walls is much higher at low temperatures. The increased viscosity probably retards the movement of water across the mass of living cells lying between the soil and the xylem of the roots. WEBER and HOHENEGGER (**30**) found the viscosity of protoplasm in the root cells of Phaseolus seedlings to be about 4 times as great at – 2.0° C. and 3 times as great at 5.0° C. as at room temperature. BĚLEHRÁDEK (**2**) cites other work indicating a general increase in viscosity of protoplasm at low temperatures and suggests that this may slow down diffusion of various substances and cause the high temperature coefficients characteristic of some biological processes. These changes in viscosity are probably concerned in the changes in permeability accompanying changing temperature which were discussed in paragraph 3.

5. The viscosity of water increases as the temperature decreases, being twice as high at 0° as at 25° C. This not only slows down the rate of movement from soil to roots but must considerably decrease the rate of movement through the root cells themselves. It is doubtless an important factor in the low water-supplying capacity of cold soils previously mentioned. The vapor

pressure of water is decreased, falling from 23.75 mm. of mercury at 25° C. to 4.57 mm. at freezing, markedly affecting diffusion and osmotic processes.

6. The physiological activity of the root cells, especially the rate of respiration, is decreased by low temperature. This would be particularly important if the absorption of water is dependent directly or indirectly upon the expenditure of energy by the root cells themselves. HENDERSON (**14**) found a correlation between the rates of respiration and absorption in roots of corn seedlings and suggested that energy needed for water intake is made available by respiration. HEYL (**15**) reviewed the literature on root pressure and presented data indicating that exudation phenomena show a marked positive response to increased temperature. This, together with other facts, led him to conclude that root pressure is probably an electro-osmotic phenomenon dependent on the respiratory activity of the root cells as a source of energy. CRAFTS and BROYER (**8**) have recently advanced an osmotic theory of root pressure which depends on the physiological activity of the cortical cells to maintain a high concentration of solutes in the xylem vessels.

Little definite evidence concerning the relative importance of the various effects of low temperature on water absorption has ever been presented. It seems, however, that in most instances decreased absorption cannot be caused primarily by decreased water-supplying capacity of the soil nor by decreased rate of root extension. Plants with their root systems in well-watered soil and in dilute nutrient solutions or tap water can be caused to wilt in an hour or less by lowering the temperature of the soil or the water to a few degrees above freezing. Root extension and water supplying capacity cannot be limiting factors on absorption under such conditions.

There has been a general tendency to ascribe the reduced absorption of water at low temperatures to decreased physiological activity of the root cells. Decreased temperatures are accompanied by decreased respiration and decreased secretory activity of the living cells, resulting in little or no root pressure at temperatures near freezing. DÖRING (**11**), for example, found that cooling the root systems of several species to 0° C. stopped bleeding or reduced it to a very low value in only five minutes. It has been found, however, that a decrease in secretory activity is inadequate to explain the decrease in absorption. BOONSTRA (**5**) observed that the rate of transpiration of peas was greatly decreased by low temperature. He decided, however, that the decrease could not have been caused by cessation of any pumping action of the root cells because he was unable to demonstrate the existence of root pressure in transpiring pea plants even at 25° C. In experiments previously described (**18**) it was found that sunflower plants grown in a culture solution and placed in potometers filled with tap water at 6° C. wilted badly within a half hour. When the roots were cut off under water the rate of absorption rose to nearly 10 times the previous rate, then fell to a new equilibrium at 2.5 times the rate prior to removal of the roots. The tops of

these plants recovered their turgor within 2 or 3 minutes after removal of their roots and remained unwilted for the duration of the experiments, or more than an hour. These results indicate that the root pressure mechanism was not playing an essential part in absorption; the wilting attendant upon cooling the roots did not result from failure of the roots to "pump" sufficient water into the tops, but from an excessively high resistance to water movement across the tissues into the xylem of the roots themselves. This high resistance must be effective in slowing down water intake regardless of the mechanism involved.

It was suggested by DÖRING (**11**) that since little or no bleeding occurs at low temperatures most of the decrease in water absorption at these temperatures must result from increased resistance to water movement across the living cells of the roots. It has been shown by the writer (**20**) that the living cells (probably principally the cortical cells) across which water passes before it can enter the xylem offer considerable resistance to water movement even at ordinary temperatures. This is probably the principal reason for the lag of absorption behind transpiration which seems to be characteristic of plants even when adequately supplied with water. It seems probable that the resistance to water movement would vary with temperature and would be much greater at low temperatures. Probably the best data on this are from an experiment by BODE (**4**) in which the rate of water movement through sunflower root systems growing in soil and attached to a vacuum pump was found to increase from 10° to 30° C. This seems to indicate that the resistance to water movement through the roots varies with temperature. In view of these facts, an extensive investigation was made of the effect of temperature on the resistance offered by the tissues of the root to water movement.

Methods and results

The sunflower and tomato plants for these experiments were grown in soil in metal containers, or in nutrient solutions, until strong stems and large root systems had developed. This required from four to six weeks. The tops were then removed near the first node and 5 ml. pipettes graduated in 0.05 ml. were attached to the stumps by rubber tubing and sealed with a mixture of paraffin, beeswax, and tallow. Enough water was added to each pipette to bring the meniscus up to the graduations on the pipette and vacuum was applied for a few seconds to remove any air bubbles.

The root systems, in their containers, were then placed in a water bath which could be maintained at any temperature from freezing to 50° C. by refrigerating and heating units. The pipettes were attached to a vacuum pump by rubber tubing and T-tubes and the pressure on the cut stems was reduced to a point where a pressure gradient of 64 cm. of mercury existed from exterior to interior of the root system. This pressure gradient was

maintained for periods of 30 minutes or one hour, depending upon the rate of absorption. By reading the pipettes at the beginning and end of the period the amount of water absorbed was accurately determined. In certain experiments similar groups of plants were prepared, but not attached to the vacuum pump. The rate of exudation at various temperatures was determined from these plants. The results given are averages of determinations on at least six plants.

In the earlier experiments a single group of plants was used for determinations over the entire range of temperatures from near freezing to as high as 40° C. It was suggested that perhaps a longer period of adjustment to a new temperature was needed than was permitted by this method and that the effects of previous exposure to low temperature might affect the behavior at high temperatures. Several experiments were then performed in which the absorption of a group of six sunflower plants was first determined at 25° C. and then at one of the experimental temperatures. These plants were then discarded and the absorption of a second group was determined at 25° C. and at some other experimental temperature. This procedure was repeated for each temperature at which determinations were to

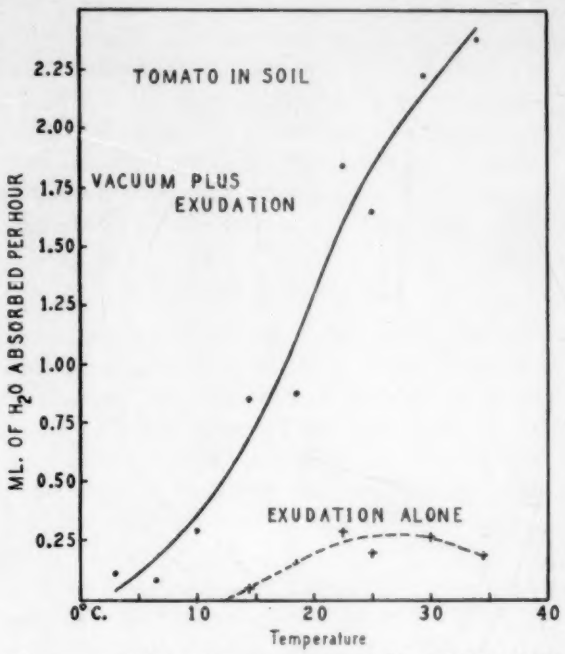

FIG. 1. Water movement in various temperatures through living tomato root systems growing in soil. Each point represents the average of six determinations. The determinations at various temperatures were made on the same plants. The upper curve is for a group of plants attached to a vacuum pump, while the lower curve shows exudation alone.

be made. Thus any one group of plants was exposed to only two temperatures; 25° C., which was approximately that at which it had been grown, and one other temperature which was higher or lower. The rates were then plotted as percentages of the rate at 25° C. The results thus obtained were similar to those obtained when one group was observed at several temperatures. The results of various experiments are much more easily compared, however, when calculated as percentages of the rate at 25° because differences caused by variations in size of root systems are eliminated. The results are shown graphically in figures 1, 2, and 3.

Figure 1 shows the behavior of tomato plants growing in soil at approximately field capacity. It will be noted that the maximum rate of exudation occurred at 22.5° C. and that the rate tended to decrease above this temperature and exudation ceased or become too slow to measure at about 12° C. The highest rate of exudation was only about 15 per cent. of the rate at the same temperature for similar plants attached to a vacuum pump. The rate of water movement at 5° C. through the roots attached to a vacuum pump was only 6 per cent. of the rate of 25° C. Increasing the temperature to 34° C. increased the rate of water movement to 143 per cent. of the rate at 25° C. In a similar experiment with sunflowers the maximum rate of exudation of plants not attached to a vacuum pump occurred at 25° C. and exudation became negligible at about 2.5° C. The highest rate of exudation was only about 17 per cent. of the rate at the same temperature for plants attached to a vacuum pump. BODE (**4**) also found the maximum rate of exudation of sunflowers at about 25° C. The rate of exudation is quite variable in individual plants, however, and the maximum rate of exudation is sometimes at 35° or 40° C. or higher.

Figure 2 shows the rate of water movement at various temperatures through living sunflower root systems in soil and in water, and through dead root systems in water. All of these root systems were attached to a vacuum pump and maintained under a pressure gradient of 64 cm. of mercury. It will be noted from the graph that the effect of temperature on the rate of water movement through living root systems in moist soil and in water was essentially the same. This perhaps indicates that roots can absorb water as readily from soil near the field capacity as from liquid water. In both instances the rate of movement was much decreased by lowering the temperature. The rate of movement through dead roots was less affected by temperature, indicating that the resistance to flow does not increase as much with lowered temperature in dead roots as in living roots. The lower curve indicates the decreasing viscosity of water with rising temperature plotted as percentages of the reciprocal of the specific viscosity at 25° C. It will be noted that the slope of this curve is approximately the same as that for the rate of water movement through the root systems. The viscosity of water

is about twice as great at 0° C. as at 25° C. and the rate of water movement through the dead roots at 1° C. is about half of the rate at 25° C. This seems to indicate that the viscosity of water may be the principal factor limiting the movement of water through the dead roots. Some additional factor must exist in the living roots which causes their permeability, and hence their resistance to water movement, to change much more with changing temperature than does the resistance to water movement of the dead roots. This additional factor probably is the change in viscosity of the protoplasm and of the colloidal gels of the cell walls. WEBER and HOHENEGGER (30) state that the

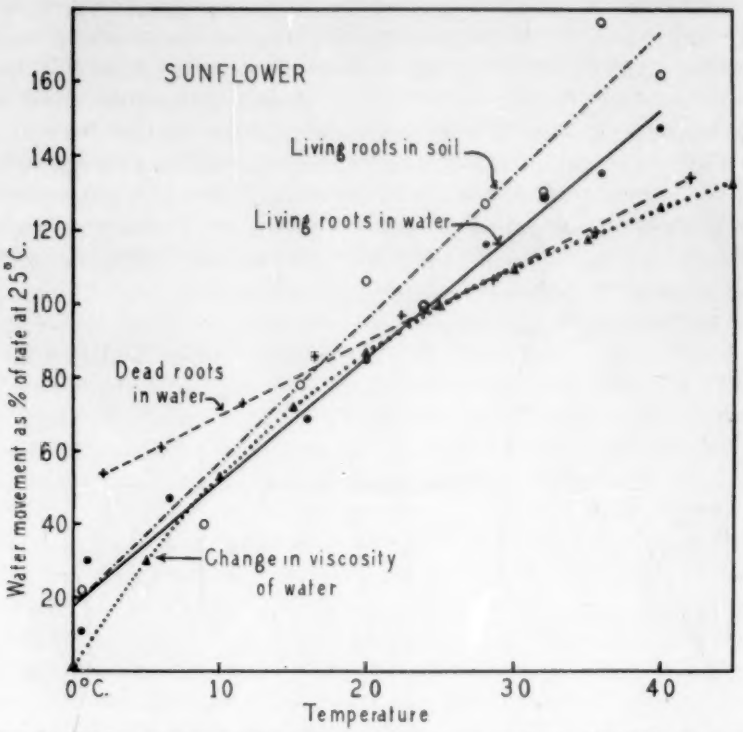

FIG. 2. Water movement through sunflower root systems attached to a vacuum pump. Plants were under vacuum for one hour and a different set of six plants was used for each temperature in all determinations on living roots. The curve for viscosity is the reciprocal of the actual viscosity, plotted as percentages of the value at 25° C. All rates in figures 3 and 4 are plotted as percentages of the rate at 25° C.

viscosity of the protoplasm of Phaseolus epicotyls is about 3 times as great at 5° C. and about 4 times as great at −2.0° C. as at 19° to 22° C. The greater increase in viscosity of protoplasm as compared with that of water probably explains the greater decrease in water movement through living root systems. Killing the cells results in collapse of the protoplasts and dis-

organization of the strands of cytoplasm passing through the cell walls, thus lessening the resistance to flow.

The results of these experiments are similar to those of BODE (**4**) who measured the rate of water movement at various temperatures from 10° to 30° C. through sunflower root systems attached to a vacuum pump. All the data available indicate a much greater reduction in water movement at low temperatures than the 25 or 30 per cent. reported by KOSAROFF (**17**). Some variations occur between different groups of plants of the same species, perhaps because of differences in heredity, past treatment, and age. Differences between species might also be expected, but the behavior of sunflower, tomato and privet was essentially the same in these experiments. It would be expected that cotton root systems which, according to ARNDT (**1**), may wilt at a soil temperature of 15° or 18° C. would show greater reduction of water movement in that temperature range than do sunflowers.

That the resistance to water movement lies chiefly in the protoplasm is indicated by the fact that much more water will pass through a dead root system than passed through the same root system under the same conditions of time, temperature, and pressure while it was alive. The rate of water movement at 25° C. through dead sunflower root systems immersed in water was 3.5 to 6 times the rate through the same root systems while alive. At 1° C. the rate was 5 to 10 times the rate through living root systems. RENNER (**23**) reported that root resistance was greatly decreased by killing the roots, and the writer (**18**) has previously reported large increases in water movement after killing the roots.

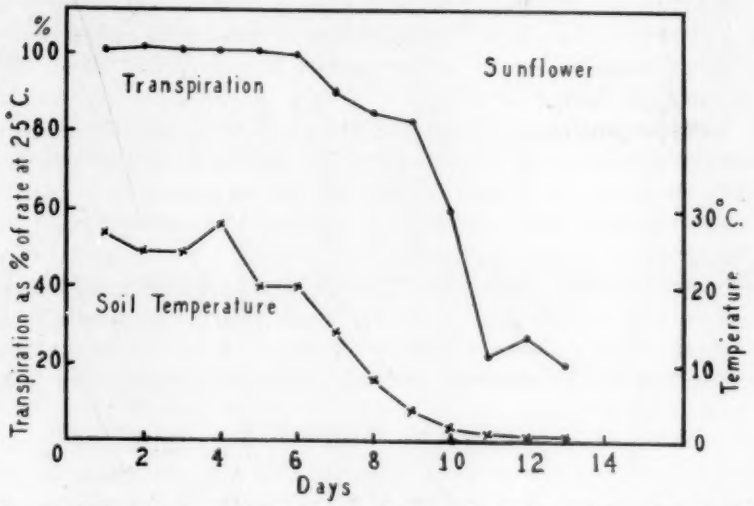

FIG. 3. Effect on transpiration of sunflowers of slowly cooling the soil. Rate of transpiration is plotted as percentages of rate of control group kept in adjacent water bath with soil temperatures of 25° C.

Figure 3 shows the decrease in rate of transpiration of a group of sunflower plants growing in soil which was slowly cooled. This was accomplished by immersing the cans of soil in which the roots were growing in a water bath which was cooled about 4 degrees daily by a refrigeration system. The rate of transpiration is given as percentages of the rate of a group of similar control plants in an adjacent tank kept at about 25° C. The light intensity and air temperatures were essentially the same for the tops of the two groups. It will be noted that at 1° C. the transpiration rate of the sunflowers fell to 20 or 25 per cent. of the rate of the controls at 25° C. This reduction agrees rather closely with the reduction in rate of water movement obtained with root systems attached to vacuum pumps as shown in figure 2. This agreement in results tends to strengthen the belief that data obtained by attaching a vacuum pump to the root system are indicative of what is occurring in the root systems of intact transpiring plants of the same species. A similar experiment was performed on potted plants of privet (*Ligustrum japonicum*). After the soil containing the root systems of these plants had been slowly cooled to about 1° C. the rate of transpiration was about 30 per cent. of the rate of the controls at 25° C.

Discussion

In considering the factors affecting the absorption of water it should be remembered that there appear to be two types of absorption occurring in many plants. These two types of absorption apparently may occur either simultaneously or independently, and their relative importance probably depends on the rate of transpiration and the internal water economy of the plant. RENNER (**22**) has differentiated these two types as "active" and "passive" absorption, but the writer prefers to term them "physiological" and "physical" absorption.

Active or physiological absorption is some sort of osmotic or secretory process, the mechanism of which cannot be explained fully at present. It is dependent on the presence of living cells in the roots and is responsible for root pressure and exudation from cut stems. Passive or physical absorption is a movement of water across the cortex along a gradient of decreasing vapor pressure from soil to xylem. This pressure gradient results from the decreased pressure or tension on the water conducting system which usually exists in transpiring plants. In these experiments the conditions bringing about physical absorption were partially simulated by attaching the root systems to a vacuum pump.

Previous experiments (**20**) have shown that at 25° C. there is considerable resistance to the movement of water across the mass of living cells lying between the epidermis and the xylem. The results of the present experiments are believed to indicate that the principal reason for the wilting of

plants whose roots are in cold soil is the reduction in physical or passive absorption caused by increased resistance to water movement across the tissues of the roots. Decreasing the temperature increases the viscosity of water, and to an even greater extent increases the viscosity of the protoplasmic membranes through which the water must pass. As a result of the increased friction less water moves into the roots with a given pressure gradient. The writer prefers to speak of the decreased water movement as being caused by changes in "root resistance" rather than by changes in permeability. Permeability is usually considered with respect to individual membranes. The intake of water is affected not only by changes in the permeability of all the membranes through which the water must pass, but also by changes in the physical properties of the water itself, and the total effect can best be described as root resistance.

The minor part in water intake played by physiological absorption and root pressure phenomena is evident when one observes that the amount of water exuding from cut stems is usually less than 20 per cent. of the amount obtained under a pressure gradient of only 64 cm. of mercury. Such a comparison is open to the criticism that removal of the top decreases the activity of the roots and hence decreases the quantity of exudation and exudation pressure. This effect is probably unimportant during at least the first two or three hours after decapitation as the rate of exudation usually does not begin to decrease for several hours. The resistance of the cortical cells is equally important regardless of whether water is being secreted into the xylem by some root pressure mechanism or moves by mass flow along a gradient of decreasing pressure caused by transpiration.

It appears that WILSON (**32**) is correct in stating that the importance of changes in the viscosity of water have been under-emphasized. STILES (**26**) points out that the temperature coefficients obtained for movement of water into various living plant tissues are considerably higher than the temperature coefficient for a physical process such as diffusion of a solute into water. DENNY (**10**) found the temperature coefficient for the passage of water through non-living seed coats to be higher at a low than at a high temperature. It was also higher than the temperature coefficient for pure diffusion. It is not surprising that the temperature coefficients for movement through such tissues and membranes do not agree with those for diffusion of solutes in water, since the conditions are much more complex in the experiments with plant membranes. The viscosity of the water, the protoplasm, and other colloidal materials of the tissues themselves are increased by lowering the temperature. All of these changes increase the friction or resistance to flow through the membranes and the combined effects will be considerably larger than for diffusion of a solute in water.

According to data of BIGELOW (**3**) the rate of movement of water through porcelain and collodion membranes at various temperatures is closely related

to the viscosity of water. In a strict sense the permeability of a porcelain membrane is probably unaffected by small changes in temperature and any change in rate of flow of water is the result of changes in physical properties of the water itself. To discuss the effects of temperature on the permeability of non-living seed coats, porcelain membranes, or dead roots probably misplaces the emphasis. Perhaps, however, we can speak of "membrane resistance" in such instances.

The effects of temperature on the rate of movement of water through various types of non-living membranes is shown in figure 4. The rates of

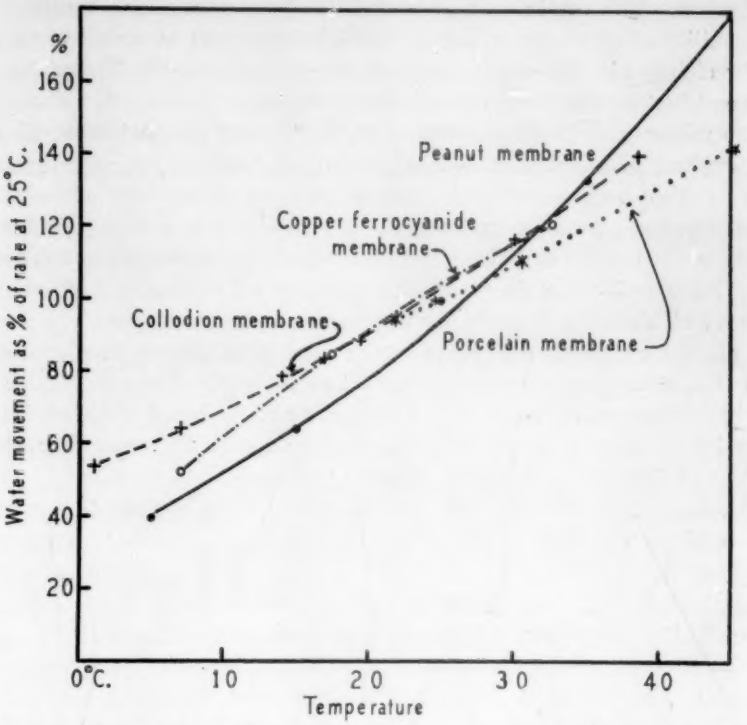

FIG. 4. Effect of temperature on rate of water movement through various types of non-living membranes.

movement are all plotted as percentages of the rates at 25° C. in order to facilitate comparison with the rates of water movement through living and dead root systems plotted in figure 2. The data for peanut seed coats are from DENNY (**11**). The data for the porcelain and collodion membranes are from BIGELOW (**3**) and are the rates of flow of water under constant hydrostatic pressure at various temperatures.

Comparison of figures 2 and 4 show that the lines for water movement by both pressure and diffusion through the mechanical membranes have the

same slope as the line for water movement under pressure through dead root systems. The line for movement by diffusion through the peanut seed coat rises somewhat more rapidly with increasing temperature and has a slope similar to that for water movement through the living root systems.

As shown in figure 2, the slopes of these lines resemble the slope of the curve for decreasing viscosity of water with increasing temperature. If viscosity of water were the only factor affecting the rate of movement of water through the membranes at the various temperatures, the lines for rate of movement should be somewhat curved to conform to the curve for the viscosity of water. It is possible that the surprisingly straight lines observed when these diverse data are plotted result from a balancing interaction between the changes in vapor pressure and viscosity that accompany changes in temperatures. Figure 5 shows the vapor pressure of water plotted as percentages of the vapor pressure at 25° C. and the curve for the recip-

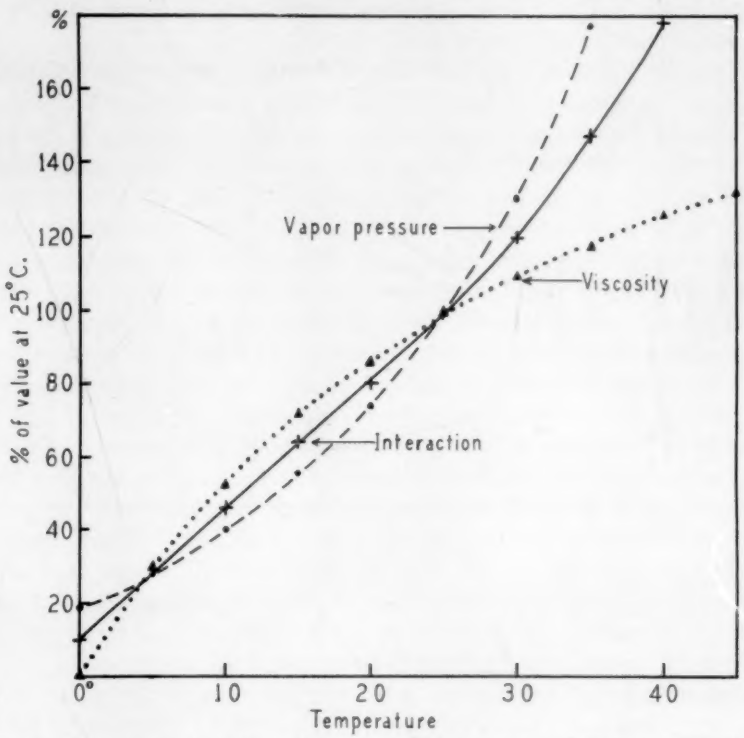

FIG. 5. Changes in viscosity and vapor pressure of water plotted as percentages of values at 25° C. The solid line is drawn equidistant between the two curves showing their possible interaction on water movement.

rocal of the viscosity plotted in the same manner. It will be observed that the line connecting points which are equidistant between the two curves is

practically straight, up to 30° C. Above 30° C. it curves upward because the increase in vapor pressure begins to occur more rapidly than the decrease in viscosity. This suggests that the straight lines obtained for the movement of water are the results of the balancing interaction of viscosity and vapor pressure. The line for water movement by diffusion through peanut seed coats definitely curves upward, especially at 35° and 45° C., as would be expected in a pure diffusion process dependent on a vapor pressure or activity gradient.

Changes in viscosity of water and especially in the viscosity of the protoplasm possibly have some effects on the absorption of minerals. HOAGLAND and BROYER (**16**) report a Q_{10} for salt accumulation by roots of 2.5 to 5.0 No doubt this results largely from the fact that salt accumulation depends on the metabolic activity of the cells. It is likely, however, that if resistance to water movement is four or five times as great at temperatures near freezing as it is at 25° C., then resistance to the movement of ions into cells is also increased by lowered temperature.

While the effects on plant growth of decreased water absorption caused by low soil temperatures have been studied frequently by ecologists and physiologists they possibly have not been considered adequately by agronomists, horticulturists, and others working on problems of plant production. According to investigations previously cited (**1, 12**), it appears that the absorption of water by cotton and sugar cane plants is checked at temperatures which are not low enough to cause wilting in sunflower or privet plants. BROWN (**6**) has recently published an interesting study of the effects of soil temperature on the growth of Bermuda grass, and Kentucky and Canada bluegrass. When the soil temperature was 21° C. and the air temperature 38° C. the leaves of Bermuda grass wilted, apparently because of inadequate absorption of water; the other two species did not wilt. Absorption of water by Bermuda grass was definitely retarded at soil temperatures of 4.4° and 10° C. while the other two species showed no wilting. It seems possible that the growth of such plants as cotton and Bermuda grass might be hindered by moderately low soil temperatures even though the air temperatures are entirely favorable for growth. One would expect this to be characteristic of most southern species but it is probably unsafe to generalize on this matter. DÖRING (**10**) studied the effect of low temperatures on the water absorption of 57 species having quite different climatic and temperature requirements; he found no consistent relationship between habitat and the extent to which low temperatures checked absorption. The data on cotton and Bermuda grass, however, indicate the possibility that the effects of low soil temperatures on the absorption of water and minerals might limit the growth of plants as effectively as low air temperatures.

Summary

1. The rates of water movement through root systems of tomato and sunflower attached to a vacuum pump were measured at constant pressure over a temperature range from 0° to 40° C. It was found in all experiments that the rate decreased with lower temperatures, both for plants in soil and in water. The rate of movement through sunflower roots at temperatures slightly above freezing averaged about 20 per cent. of the rate through the same roots at 25° C. and the rate at 40° C. averaged about 160 per cent. of the rate at 25° C.

2. The rate of exudation from root systems not attached to a vacuum pump was highest at about 25° C., decreasing with both increased and decreased temperature. The rate became too low to measure at about 12° C. in tomatoes and 2.5° C. in sunflowers. The highest rate of exudation was less than 20 per cent. of the rate obtained with the same or similar plants attached to a vacuum pump under a pressure gradient of 64 cm. of mercury.

3. The rate of movement of water through dead sunflower roots attached to a vacuum pump also decreased with decreasing temperature, but not to the same extent as in living roots. The rate at 0° C. was about 50 per cent. of the rate at 25° C. and the rate at 40° C. was 130 per cent. of the rate at 25° C.

4. It is believed that the decreased rate of movement of water through dead roots at low temperatures is largely caused by the increased viscosity of water itself. In living roots the added effects of decreased permeability, probably resulting from increased viscosity of the protoplasm and of the colloidal gels of the cell walls, causes an even greater resistance to water movement. The resistance to water movement through living roots of sunflower appears to be four or five times as great at temperatures near freezing as at 25° C. At 40° C. the resistance is only a little over half that at 25° C.

5. The principal cause of decreased water absorption by plants at low temperatures appears to be the combined effects of decreased permeability of the root membranes and increased viscosity of water, resulting in increased resistance to water movement across the living cells of the roots. The effects of low temperature in decreasing root extension, root respiration, active absorption and root pressure phenomena are of secondary importance. The increase in root resistance with decreasing temperature probably is effective, however, in slowing down physiological, or active, absorption of water, and possibly the absorption of solutes by roots.

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SEASONAL VARIATIONS IN THE PRODUCTION OF PLANT PIGMENTS

WILLIAM A. BECK AND RICHARD REDMAN

(WITH FOUR FIGURES)

Introduction

The production of the pigments chlorophyll, xanthophyll, and carotene in green plants apparently depends upon the structure of the colloid carrier, the chloroplast (**30**), and upon the activity of the protoplasm in which the chloroplast is immersed. The favorable physico-chemical state has been termed chloroplastinsymplex (**3**). Production of the pigments probably depends upon the organization of the protoplasm and may be termed a vital or physiological process which involves the activity of certain obscure internal factors. It makes little difference in our present study whether it is assumed that these obscure factors are purely physical or chemical, thus involving no other laws than those that are already known to physicists and chemists; or whether, on the other hand, it is assumed that they involve entities that lie beyond our present knowledge of matter and energy. The organization of the protoplasm which is implied in the use of the term "physiological" will hardly escape any careful observer.

It appears that the production of these pigments depends upon certain factors, some of which are internal and others external. The effects produced by certain external factors may be direct, or they may be indirect, in so far as they affect the "organization" of the protoplasm, which itself may be immediately responsible for the formation of pigment.

The effect of some external factors upon the production of pigments has been studied in plants under controlled conditions in our laboratories (**3, 5, 6, 7, 8, 9, 10**). It was found that light favored the production of all three pigments; while it was essential for chlorophyll production, the carotenoid pigments could develop to a limited extent in etiolated seedlings, from reserve materials contained in the seed, without the aid of light. Carotene and xanthophyll probably form a respiratory mechanism for the protoplasm in the seedling (**3**). Carotene may even be important for the early growth of the seedling, functioning as a "sensitizer" (**5, 27, 30**). LAZAR found that carotene (pro-vitamin A) increased the number of roots formed in the cuttings of Impatiens (**16, 17, 18**). That some relationship exists between carotenoid pigments and growth promoters is indicated by the fact that the absorption spectra of these pigments coincide closely with the spectral distribution of the phototropic sensitivity of the Avena coleoptile and the sporangiophores which normally contain these pigments (**11, 12**). PFEFFER (**24**) suggested that the absence of chlorophyll in etiolated plants might be due to some disturbance of the nutrition complex.

The three pigments develop well in the atmosphere of compressed air, at least for a limited period of time, but not in compressed carbon dioxide (**3, 7**). The development is roughly proportional to the general metabolism. This is in agreement with the notion that external factors influence the development of pigments because they affect the "organization" of the protoplasm. It did not appear probable from our results that slight changes, such as normally occur seasonally in the pressure of the atmosphere, would seriously affect pigment production in plants.

When the illumination was not particularly intense it was found that the chlorophyll produced was proportional to the quantity of light received by the plant (**9**). Apart from a possible temporary initial decline in the carotene present in etiolated plants, (**5, 10, 23**) the production of this pigment was also roughly proportional to the quantity of light received when it was not too intense. Xanthophyll production followed the same rule (**7**).

Other investigators show that the wave length of light employed is an important factor in the process of pigment production (**14, 15, 25, 28**). Seasonal variations in the pigment content of leaves might well be expected since the significant factors which influence the production of pigments in plants vary considerably during a year. These factors are the wave length of the radiation and the intensity of the light and heat; it is probable that the intensity of light and the temperature are factors of greater significance than the wave length of light or the moisture conditions.

Accumulation of pigment depends not only upon the amount produced, but also upon the possible destruction of the pigments by such factors as, for example, excessively intense radiation. Such harmful effects may be produced directly or as the result of partial disorganization of the activity of normal internal factors. LUBIMENKO claims that there is an optimum of light in this respect, and that it varies with the species and the age of the plant. The experiments of SHIRLEY (**29**) also point to such an optimum. From this it seems quite natural that shaded plants might well produce more pigments than plants exposed to intense sunlight. The experiments of BECK (**8**) show that this is actually true in certain cases. It is difficult to say if the results obtained by him are entirely due to the intensity of the light, as such, or if the heat factor involved is largely responsible. It is very probable that the heat plays a considerable rôle. HENRICI (**13**) found that the light affected differently the chlorophyll production in different kinds of plants, and also affected differently the chlorophyll production in older and younger leaves. In general, plants with high chlorophyll content show greater variation with change in illumination than do those with low content, which would make it appear as though the light does not act immediately in the production of the pigment.

LUBIMENKO and HUBBENET (**22**) studied the relationship between temperature and chlorophyll production. They concluded that the increase in

production of the pigment in light is conditioned by temperature, because additional synthesis of the precursor of chlorophyll depends upon the temperature. The minimum temperature was found to be between 2° and 4° C., the optimum 26° C., and the maximum 48° C. It is interesting that these values agree roughly with the respective values of the temperature which produces variation in the osmotic value of the cells of assimilating tissues in plants. If it is assumed that the osmotic value of the assimilating tissues is an indicator of the metabolic activity of the tissue (**1**) then it may be stated that the production of pigment present in the tissue is proportional to the metabolic activity induced by the influencing physical factors of the environment, particularly the intensity of light and the temperature. This follows from what was proved to be true for pigment production under controlled conditions and from the effect of light and temperature on the osmotic value of the assimilating tissues (**2**).

After working with external factors under controlled conditions we proceeded to study the seasonal variations of pigment production under normal conditions. Our work extended over a period of almost two years from August 11, 1936, to May 13, 1938. The study of xanthophyll and carotene was discontinued after the completion of the first cycle but the study of chlorophyll was extended through both.

Methods

Dutch Sweet Clover was chosen as our test plant because it did not freeze readily and was abundant in our large open lawn, where no shadows struck the plants and where they were rarely disturbed. Tests were usually made monthly; they were made more frequently, however, when rapid variations of pigment content were indicated.

The older method of extracting the pigment from dried material was not employed since losses through alcoholysis and changes in the chlorophyll are often experienced when using this method. The pigments were extracted by the methods indicated by SCHERTZ (**26**). BECK's device was employed for the quantitative determination of the pigments (**4**). Two hundred leaves (600 lobes) were taken for each test. The green weight was determined before the extraction was made, and the weight of the dry pulp was determined after the extraction was completed. The three pigments, whose qualities are recorded for a given test, were obtained from the same lot of plants.

The light factor in arbitrary units was obtained by multiplying the average of the number of hours of sunshine reported per day for the period by a correction factor. The factor was unity for the period from March to September. For October, November, and January it was two-thirds and for the month of December, one-half. This expression for the light factor

was a fair approximation of what would have been obtained if we had calculated the efficiency of the light per square inch brought by a ray falling perpendicularly upon a horizontal surface, multiplied by the sine of the angle which the actual beam made with the horizontal, and had multiplied the number of hours of sunshine by the figures thus obtained. That our figures were not far from the real values may be seen from the graphs of light intensity and temperature which were taken directly from the record. They are reasonably well in agreement (fig. 2).

Results and discussion

Results are recorded in table I. The highest values recorded for the three pigments were as follows: chlorophyll 43.5 mg., xanthophyll 3.480 mg., carotene 1.173 mg.

TABLE I

PIGMENT CONTENT OF DUTCH SWEET CLOVER PER 200 LEAVES AND THE VALUES OF THE INFLUENCING FACTORS, LIGHT AND TEMPERATURE, FROM AUGUST 11, 1936 TO OCTOBER 20, 1937

DATE	CHLOROPHYLL	XANTHOPHYLL	CAROTENE	LIGHT FACTOR IN ARBITRARY UNITS	AVERAGE TEMPERATURE
	mg.	*mg.*	*mg.*		°*F.*
8–11–36	16.375	2.915	0.680	11.36	77.2
9–14–36	22.000	1.790	0.642	9.60	79.4
10–14–36	23.250	1.598	0.684	4.99	64.8
11–19–36	16.245	1.800	0.461	3.57	47.4
12–14–36	14.200	1.350	0.360	2.60	33.7
1–12–37	12.600	0.906	0.264	2.56	41.0
2– 9–37	9.970	0.954	0.345	2.35	35.8
3– 2–37	7.540	0.954	0.306	5.33	33.9
3–18–37	5.550	0.954	0.303	6.14	36.4
3–31–37	11.100	1.725	0.435	7.32	41.3
4–13–37	15.720	1.725	0.558	6.23	46.8
4–27–37	31.600	3.480	1.173	6.66	57.0
5–13–37	34.200	2.068	0.684	9.24	58.2
5–20–37	43.500	.....	.....	9.46	57.6
6–15–37	23.600	0.940	0.234	14.95	72.4
7– 8–37	26.750	0.895	0.586	13.65	73.4
7–27–47	21.100	0.920	0.586	15.21	76.2
9– 9–37	24.600	1.880	0.684	10.06	76.2
10–20–37	19.300	2.385	0.729	4.91	59.0

The study of pigment ratios proved interesting. The ratios are recorded in table II. The order of the numbers expressing the relative quantities is the same as that usually found in such studies. The amount of chlorophyll at one time was 98 times as great as that of the carotene, but on an average it was 36.2 times as great. It was 29 times as great as the xanthophyll at one time but on an average only 12.5 times as great. The amount of xanthophyll

TABLE II

PIGMENT RATIOS FOR THE YEAR 1936–1937

DATE	CHLOROPHYLL: XANTHOPHYLL RATIO	CHLOROPHYLL: CAROTENE RATIO	XANTHOPHYLL: CAROTENE RATIO
8–11–36	5.6	24.0	4.2
9–14–36	12.3	34.0	2.7
10–14–36	14.6	34.0	2.3
11–19–36	9.0	35.0	3.9
12–14–36	10.0	39.0	3.8
1–12–37	13.9	47.0	3.4
2– 9–37	10.4	28.0	2.7
3– 2–37	8.0	24.0	3.1
3–18–37	5.8	18.0	3.1
3–31–37	6.4	25.0	3.9
4–13–37	8.9	28.0	3.1
4–27–37	9.0	27.0	2.8
5–13–37	16.5	50.0	3.0
5–20–37	.....	.....	.....
6–15–37	23.0	98.0	4.0
7– 8–37	29.0	44.0	1.5
7–27–37	22.0	35.5	1.5
9– 9–37	13.1	35.5	2.6
10–20–37	8.0	26.0	3.1
AVERAGE	12.5	36.2	2.9

was never more than 4.2 times as great as that of the carotene and at one time only 1.5 times as great. The average ratio was 2.9.

The regularity with which the ratios change in the different seasons is significant and suggests some relationship of the pigments, if not in their origin or the building of their molecules, at least in their functions. The slight variation of the xanthophyll : carotene ratio is particularly significant. Xanthophyll and carotene appear to be relatively more abundant at those times immediately preceding a growth period; this is in agreement with the notion that carotenoids serve, directly or indirectly, as growth promoters (**3, 5, 16, 17, 18**).

Water makes up the major part of the green weight as was verified from the weight of dry pulp taken at the conclusion of each extraction. From a comparison of the green weight and the prevailing conditions it would appear that relative humidity, soil moisture, temperature, and light influenced the amount of water absorbed. Our results are in agreement with those obtained by URSPRUNG (**31**) for the seasonal variations of suction tension in plants if we take for granted that high suction tension is interpreted as low water content and *vice versa*. URSPRUNG's data showed that from June, 1925 to June, 1926 the suction force of Bellis for a given month depended on the distribution, as well as the quantity, of precipitation.

In figure 1 is shown graphically the comparison of green weights and

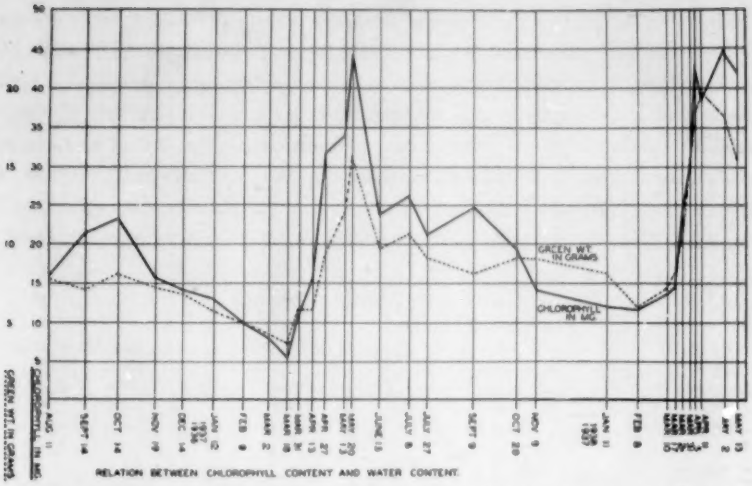


FIG. 1. The relation between chlorophyll content and water content.

chlorophyll yield during the two years. It is evident at once that the general trend of chlorophyll production and water content (assuming that variations in water content are reflected in the variations in green weight) are the same. The predominant factors (light and heat) probably affect both to about the same degree. Less effective factors such as moisture conditions may affect the one somewhat more than the other, producing local deviations in the direction of one graph from that of the other. The chlorophyll production probably depends in a secondary manner upon the water taken up by the plant, but the suction tension of the plant depends upon the moisture of the soil (**31**). A notion of the soil condition may be obtained from the total precipitation for each month.

Comparing the figures for the winter of 1936–1937 with those for the winter of 1937–1938, it must be remembered that the former winter was relatively wet (January, 1937 was the month of the great flood) and the latter relatively dry. The total precipitation values in inches for November, December, and January for the former were 4.09, 3.36, and 14.08, respectively; for the same months of the latter 1.33, 3.54, and 1.46, respectively. The favorable moisture conditions did not favorably affect green weight and pigment production because the temperature was unfavorable in the colder winter of 1936–1937. The spring of 1938 was considerably more wet than the spring of 1937. Precipitation figures for February, March, and April were as follows: 1.22, 1.05, and 2.48 in., respectively, for the same months in 1938, 2.25, 6.41, and 1.69 in., respectively. The favorable temperature permitted the effect of soil moisture to express itself immediately in favor of both the green weight and pigment production (fig. 1). On April 11, 1938 a secondary low is noted for pigment even though the green weight

is at a peak. No such difference is noted for the previous year. The spring of 1937, though relatively dry, had a steady increase in precipitation; in the spring of 1938 the fluctuations of the precipitation were great. In 1938 the temperature dropped from 56° F. through the greater part of March to 42° and 45° F. in April, after which it rose to 63° F. in May. The temperature variation is clearly reflected in the graph for the chlorophyll but not so definitely in the graph for the green weight which was favored somewhat more by the relatively moist soil in the spring of 1938. This clearly shows that while soil moisture does actually favor chlorophyll production it is far less important as a factor than is light or temperature.

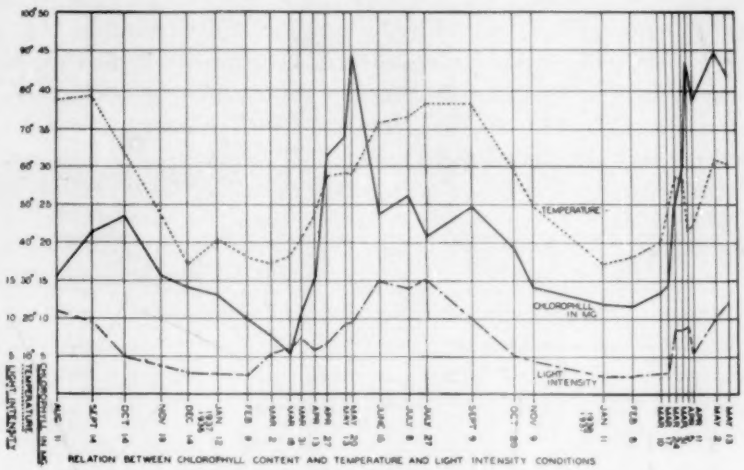
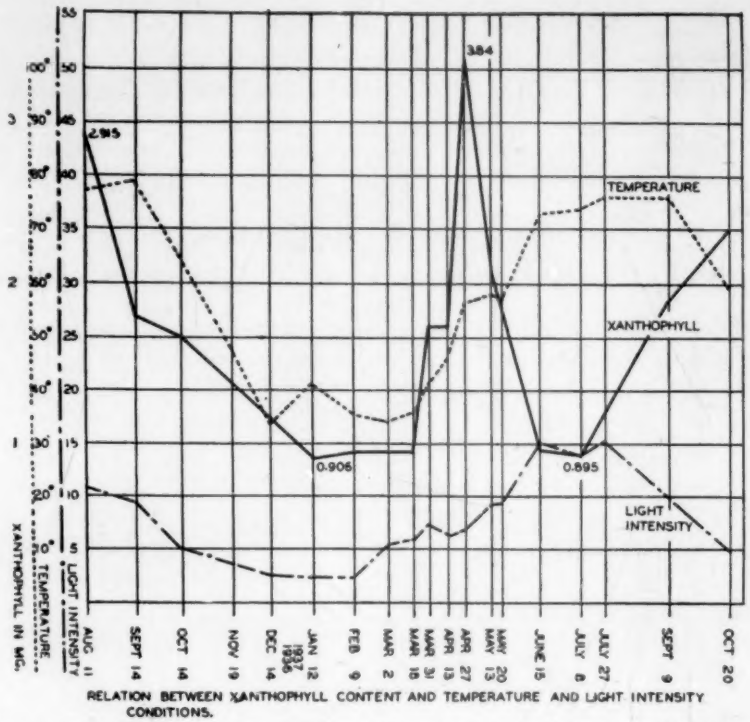

TEMPERATURE
CHLOROPHYLL IN MG.
LIGHT INTENSITY
RELATION BETWEEN CHLOROPHYLL CONTENT AND TEMPERATURE AND LIGHT INTENSITY CONDITIONS

FIG. 2. The relation between chlorophyll content, temperature, and light.

The graphs showing relationships between chlorophyll production and temperature and light are given in figure 2. Similar relationships for xanthophyll and carotene are given in figures 3 and 4.

All three pigments had peaks in the same seasons, spring and fall, and their lowest points in winter and summer (table I and figs. 2, 3, 4). It is particularly interesting that in the spring of 1937 the chlorophyll reached its peak on May 20 but the xanthophyll and carotene had already reached their peak a month before (April 27). In the seedlings studied, under controlled conditions the carotenoids developed in the dark before chlorophyll could develop. This makes the phenomenon of the appearance of carotenoids before chlorophyll more general and gives it more heuristic significance. Since there is some relationship between the carotenoid pigments and growth promoters (**11, 12, 16, 17, 18**) it is not surprising that they should appear in increased quantity in seedlings and mature plants shortly before the time when the plants are developing assimilating tissues in which the chlorophyll

will appear and the plants will avail themselves of those factors in the environment which are particularly favorable for photosynthesis. Factors that favor the development of chlorophyll are evidently considerably less favorable for the development of the carotenoid pigments. The decline in the yield of these pigments later was very marked when the chlorophyll was still increasing in quantity (fall of 1936). The carotenoids were also on the decline when the chlorophyll was in the ascendency. In the fall of 1937 things were not the same since the carotenoids were still increasing even after the chlorophyll had passed its secondary peak on September 9. The summer had been unusual, with drought conditions from June to September. The unusual climatic conditions of the spring of 1937 probably caused the slight difference in the general trend of the three pigments from that of the spring of 1936. In a separate series of experiments it was shown that unusually high heat and drought produce a decline in the carotene yield, at least temporarily (**10**). In 1937 the peak for the chlorophyll (fig. 2) was reached on May 20 at 43.5 mg. and in 1938, on May 2, at 44.8 mg. The difference is negligible. There was, however, a temporary decline between



RELATION BETWEEN XANTHOPHYLL CONTENT AND TEMPERATURE AND LIGHT INTENSITY CONDITIONS.

FIG. 3. The relation between xanthophyll content, temperature, and light intensity conditions.

April 5 and the date of the peak which is readily explained by corresponding changes in temperature and light. The predominant effect of the light and temperature throughout the period is evident.

Chlorophyll

The light factor is quite similar to temperature but is predominant. Ten (arbitrary units) is the optimal value of the light factor (fig. 2). This value was obtained on May 20, 1937 at the time of the chlorophyll peak. On June 15, it rose to its peak (15) and the chlorophyll declined; it then returned to peak on July 27 and the chlorophyll again declined to a point even lower than on June 15. This additional decline was undoubtedly caused by the simultaneous rise of the temperature to its peak, which was considerably above the optimum. In spite of the continued high temperature, the chlorophyll rose (July 27 to September 9) because the light factor had returned to the optimum of 10. The chlorophyll could not rise to the value of the primary peak because the temperature was too high. On October 20 the light factor was only 5, the temperature below optimum, and thus the chloro-

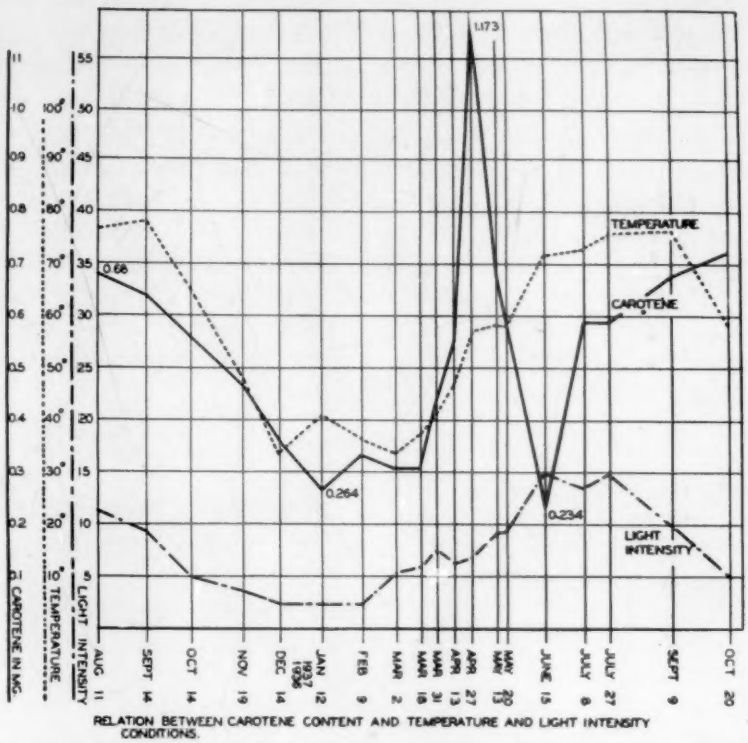


Fig. 4. The relation between carotene content, temperature, and light intensity conditions.

phyll moved into the winter decline. The low was reached February 8, 1938. The light factor and the temperature were about the same at that time, as they were December 14, 1936, on which day the chlorophyll reading was just a little higher. Probably some secondary factors (*e.g.*, soil moisture) were responsible for the difference. The precipitation during the period preceding December 14, 1937 was greater than that during the period preceding February 8, 1938; this accounts satisfactorily for the higher value of the chlorophyll content on December 14, 1937, in spite of the fact that the temperature and light factors were approximately the same on the two dates.

January of 1937 was warmer (flood year) than the January of 1938, but the light factor was about the same; in agreement with these conditions the chlorophyll content was somewhat higher on the first date. The low of March 18, 1937 (7.54 mg.) is considerably below that of February 8, 1938 (11.45 mg.). The drought of February and early part of March, 1937 were probably responsible for this deeper low extending over into March. The rises from low to peak were rapid in both years. The influence of the light factor and temperature is particularly well expressed from April 5 to May 2 in 1938. The chlorophyll yield fell temporarily with light and temperature but rose with them until the peak of the previous year was exceeded by 1.3 mg.

Xanthophyll

The variations of yield of xanthophyll with the variations of light and temperature are shown graphically in figure 3. The peak was reached April 27 at which time the light factor was 6.66 and the temperature was 57° F. Apparently this was the optimum combination of the two factors. It ran quickly to a low as the intense light and heat of summer set in, rising again in fall with the decline of heat and light. The low of January (0.906 mg.) was almost the same as that of summer (0.895 mg.). It is particularly interesting that xanthophyll and chlorophyll do not develop in exactly the same way in response to the radiation factors.

Carotene

The relationships between the development of carotene and light and temperature are shown graphically in figure 4. The peak is reached at the same time as that of xanthophyll, April 27. The optima of the radiation factors are presumably about the same for the development of both pigments. Here also the summer low (July 8) 0.234 mg. is still lower than the winter low (January 12) 2.64 mg. Like the xanthophyll, it was still rising from September 9 to October 20, 1937, while it was falling from August 11 to October 14, 1936. The light factor and temperature conditions were different in the two years and, interpreted from the point of view of the optimum combination, the recorded values are just what might have been expected.

In the fall of 1936 at the times given, the light factors were 11.36 and 9.6 and the temperatures were 77° and 79° F.; in the fall of 1937, however, they were more favorable for carotenoid development, *i.e.*, light factors 10 and 4.9 and temperatures 76° and 59° F.

Summary

1. From the results obtained in studies of pigment yield in plants raised under controlled conditions it appeared probable that seasonal variations would occur in the amount of pigment produced under normal conditions.

2. There were evident and regular variations. The factors may have exercised their influence directly or by affecting the organization of the protoplasm; the latter notion is the more probable. The general trend of variations was the same for all three pigments. A close relationship was revealed between the variations in green weight and the amount of pigment present which suggested that both depended upon the organization of the protoplasm.

3. The three pigments showed a primary peak in their yields in spring and a secondary one in fall. There were corresponding lows in winter and summer. The times of peak and low were about the same for xanthophyll and carotene but the time of peak for the carotenoids preceded that for chlorophyll. The yield of chlorophyll reached its primary peak early in May and the secondary peak in September. The primary low was in the early part of March and the secondary early in July.

4. There was always considerably more chlorophyll present than carotenoid pigment. On an average there was 12.5 times as much chlorophyll as xanthophyll and 36.2 times as much as carotene. There was 2.9 times as much xanthophyll as carotene present on the average. The ratios varied considerably for chlorophyll and the carotenoids but very little for xanthophyll and carotene. In all cases the variations occurred in a remarkably regular manner which suggests a relationship of the pigments, if not in their origin, at least in their functions.

5. The fact that the carotenoids develop vigorously immediately before the growing season is in agreement with the notion that they act, directly or indirectly, as growth promoters.

6. Light and temperature were evidently the factors which most influenced pigment production. Soil moisture played a minor rôle.

7. There is an evident optimum light intensity and also an optimum temperature. Before these optima are reached the production of pigment increases with light and temperature but beyond the optima, production is greatly reduced. These optima are about the same as the corresponding optima for metabolic activity, as deduced from the O_g values of the cell. This suggests that in order to obtain maximum activity and maximum yield

under controlled conditions, the control of light and temperature is as important in summer as it is in winter.

The authors are indebted to the Abbe Meteorological Observatory, Cincinnati, Ohio for their meteorological data and wish to express their thanks for the kind cooperation of its staff.

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CINCINNATI, OHIO

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EFFECTS OF BORON DEFICIENCY AND EXCESS ON PLANTS

SCOTT V. EATON

(WITH SEVEN FIGURES)

Introduction

There is a large literature in regard to the effects of boron deficiency on plants. Boron is now commonly regarded as an essential element for the growth of plants. Most of the literature demonstrating this fact has appeared during the last 15 years, although certain French investigators, especially BERTRAND, AGULHON and MAZÉ, showed the beneficial effects of boron on the growth of plants much earlier than this. BRENCHLEY (**2**) reviews this early literature.

In England, BRENCHLEY and WARINGTON (**1, 19**), and in America, SOMMER and LIPMAN (**17**) early showed the favorable effects of boron on the growth of various plants. No attempt is made to review the boron literature. MCMURTREY (**14**) gives a rather recent summary of it. It might be expected that an element needed in such small amounts (0.5 p.p.m. is usually sufficient and may be excessive) would never be lacking in the soil. But cases of deficiency under natural conditions have recently been reported (**9, 13**).

There is also a large literature dealing with the toxic effects on plants of excessive amounts of boron in natural conditions in the soil and in irrigation water (**5, 16**), in fertilizers (**15**), and in nutrient solutions used experimentally (**11, 19**). Much of the earlier literature in regard to the toxic effects of boron is reviewed by BRENCHLEY (**2**).

Although there is a large literature on the effects of boron deficiency and excess in the growth of plants, few investigations have used the sunflower. Since the results of the present investigation are rather striking, it is thought worth while to place them on record.

This paper has developed from work done on the effects of sulphur deficiency on the metabolism of plants (**7**). In this investigation as described below boron deficiency developed; so experiments were performed to determine the proper concentration of boron to use in these sulphur experiments. These experiments were extended into the toxic realm. The methods were essentially the same as those already described (**7**). Chemicals of C.P. or reagent quality were used, but no attempt was made to further purify them. In the case of the plus-boron cultures, boron was supplied in the form of boric acid. The plants were grown in pure quartz sand, which was given no other treatment than that of flushing it out thoroughly with distilled water before the seed was planted. Somewhat different results might have

been obtained if the chemicals and the sand had been further purified. Most of the data have to do with the sunflower,[1] but in the case of boron deficiency a few data are recorded for the soy bean.

Experimental data

Boron deficiency

Soy bean.—In the spring of 1933, a study was made of the effects of sulphur deficiency on the metabolism of the soy bean (7). The Manchu variety of soy bean was grown in sand culture. About 5½ weeks after the planting of the seed many of the plants had dead tips. Boron in the form of boric acid was applied at a concentration of 0.5 p.p.m. In a short time side shoots were put out. The early symptoms of boron deficiency were not observed, because it was not expected that boron would need to be supplied. In other work, a different variety of soy bean, Wisconsin Early Black, had been grown for 5 weeks without any symptoms of boron deficiency developing (6).

In the autumn of 1936, the Manchu variety of soy bean was grown in sand culture in the greenhouse in order to get more definite symptoms of boron deficiency in the soy bean. The same methods were used as in the 1933 experiment. There were three pots each of plus and minus boron plants. Boron at a concentration of 0.5 p.p.m. was applied twice a week when the pots were flushed out with distilled water. The seed was planted on October 3. By November 18, 46 days from the time of planting, definite symptoms of boron deficiency had developed. The main symptom noticed at this time was a whitening between the veins of the young leaves, the veins themselves appearing much greener than the interveinal areas. By December 8, 66 days from the time of planting, boron deficiency symptoms were quite pronounced. The main symptoms were: dying of the tips, the crinkled character of the upper leaves, the curling downwards of the tips of the young leaves, and the lack of greenness as compared with the plus-boron leaves. The lack of greenness was manifest especially by white lines between the veins of the young leaves, the veins themselves including narrow areas on each side which were much greener. White spots or splotches also developed on the upper leaves. The minus-boron plants were of course much shorter than the plus-boron. Figure 1 gives some idea of the appearance of the leaves; figure 2 compares the height of the plants of the two series. Table I gives the weights of the plants. The plants were harvested on December 8. The data are for 10 plants, but there were 14 plus-boron and 13 minus-boron plants. It is realized that the plants were rather few in

[1] Results quite similar to those recorded in this paper were obtained by Mr. J. C. Frazier in the summer of 1935 in class work under the direction of Dr. Charles A. Shull. He used the water culture method, and showed the effects of both deficiency and excess of boron on the growth of the sunflower.

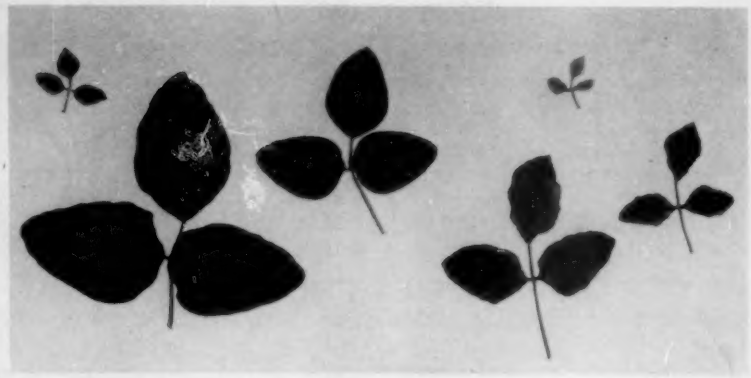

FIG. 1. Young leaves of soy bean plants, December 7, 1936, 65 days from planting of seed: right, plants receiving no boron; left, plants receiving boron at a concentration of 0.5 p.p.m.

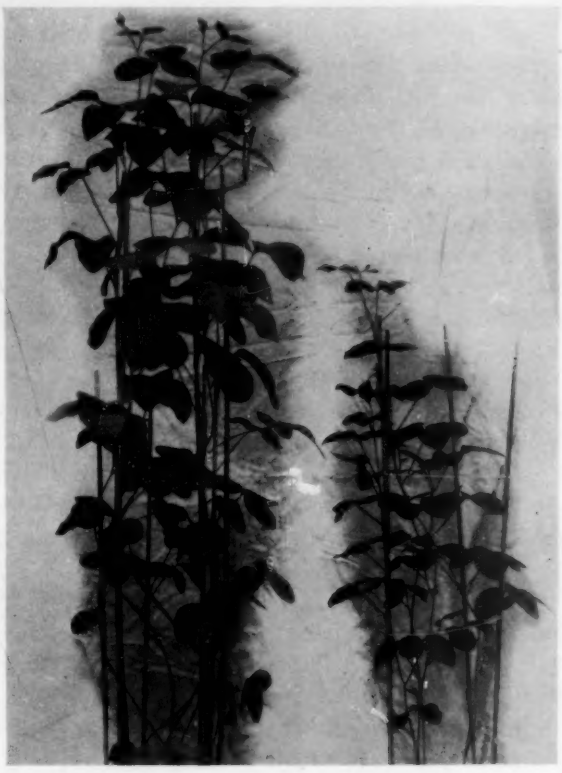

FIG. 2. Soy bean plants, December 5, 1936, 63 days from planting of seed showing relative height: right, receiving no boron; left, receiving boron at a concentration of 0.5 p.p.m.

TABLE I

EFFECT OF BORON DEFICIENCY ON GROWTH OF SOY BEAN

BORON	GREEN WEIGHT PER 10 PLANTS				
	LEAVES	STEMS	TOPS	ROOTS	ENTIRE PLANT
p.p.m.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0.0	54.69	41.92	96.61	8.84	105.45
0.5	128.78	73.21	201.99	19.42	221.41

number, but the differences are so great that the results are certainly significant.

It would seem that the soy bean needs a rather small amount of boron for normal growth. Boron at a concentration of 0.5 p.p.m. applied twice a week is certainly sufficient. In fact, in the spring of 1934 (7), normal plants were secured by applying twice a week boron at a concentration of 0.25 p.p.m.

SUNFLOWER.—Considerable preliminary work has been done in a study of the effects of sulphur deficiency on the metabolism of the sunflower, variety Mammoth Russian. One phase of this work was the determination by the triangular method (10) of the best concentration of the salts of the nutrient solution. The plants were grown in sand culture in the spring of 1935 by the same methods as have been described for the soy bean. There were 21 combinations of the salts, and each combination was run in triplicate. Because boron deficiency had developed in the long growing of soy beans, as previously mentioned, it was planned to add boron during the later stages of growth. In less than 4 weeks, however, the upper leaves of many of the plants had assumed an unhealthy appearance. They were crinkled and mottled, and resembled a good deal mosaic infected leaves. In fact, at first the trouble was diagnosed as mosaic, and unsuccessful attempts were made to infect with extracts of these leaves healthy leaves of plants growing in soil. Later it was found that the symptoms were those of boron deficiency but this was not thought of at the time because the trouble had developed so soon and because the symptoms differed somewhat from those of boron deficiency in the soy bean.

Since it was evident before the experiment was completed that the trouble above described was caused by boron deficiency, it was decided to try an experiment with the object of determining the best concentration of boron for optimal growth of the sunflower. The concentrations were extended into the toxic realm in order to determine the nature of the toxic effects of this element on the sunflower. Figure 3 and table II give the concentrations used. The experiment was tried in the autumn of 1935 and was repeated in the spring of 1936. The same methods were used as in the sunflower

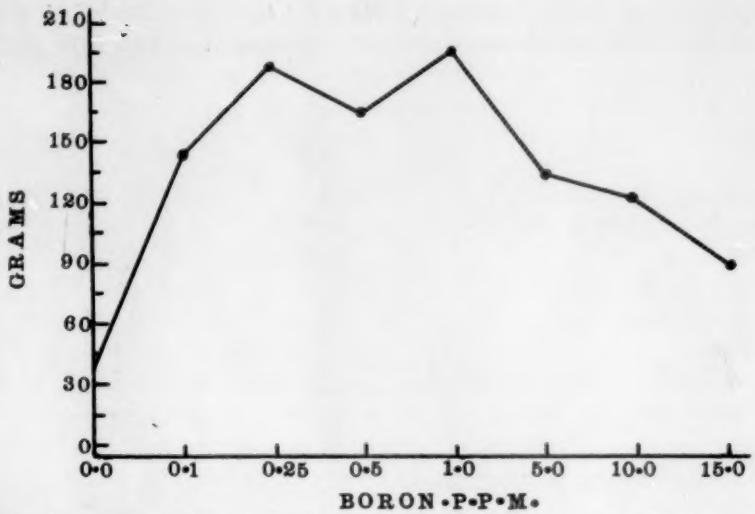

FIG. 3. Green weight per 10 plants of sunflower plants, supplied with various amounts of boron, November 25, 1935, 53 days from planting of seed.

experiment above described. The seed for the 1935 experiment was planted on October 3 and the plants were harvested on November 25; in 1936 the seed was planted on April 3, and harvesting was done on May 22 and 23. Table II gives the weights of the plants of the two experiments, and figure

TABLE II

EFFECT OF VARIATION IN CONCENTRATION OF BORON ON GROWTH OF SUNFLOWER

Boron	Green weight per 10 plants					
	Autumn, 1935			Spring, 1936		
	Tops	Roots	Entire plant	Tops	Roots	Entire plant
p.p.m.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
0.0	38.5	1.25	39.75	439.83	70.0	509.83
0.1	144.0	6.4	150.4	740.66	142.58	883.24
0.25	188.35	9.58	197.93	700.58	152.91	853.49
0.5	165.2	4.5	169.7	759.49	190.83	950.32
1.0	196.0	7.54	203.54	878.63	207.27	1085.9
5.0	135.08	5.16	140.24	512.57	105.21	617.78
10.0	123.09	4.54	127.63	509.41	100.50	609.91
15.0	90.58	3.90	94.48	531.99	86.54	618.53

3 represents graphically the weights of the plants of the 1935 experiment. The weights are for 10 plants, but the actual number of plants ranged from 8 (only one series less than 10) to 12 in the 1935 experiment and from 11 to 18 in 1936. Though the plants are few, the weights differ so decidedly

that they are no doubt significant. Figure 4 compares the height of the plants of the 1936 experiment up to and including the series 5.0 p.p.m.

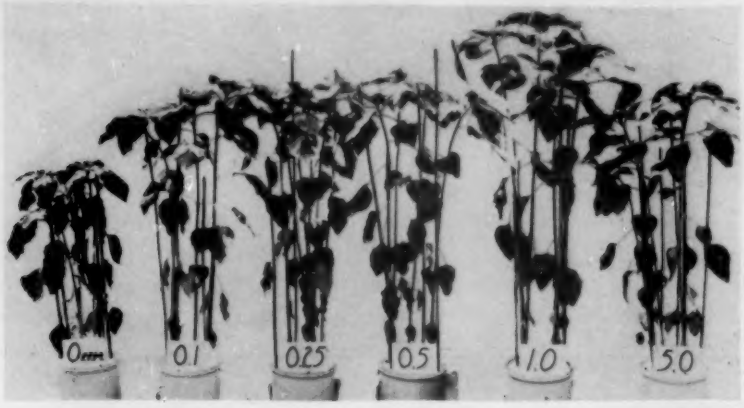

FIG. 4. Relative height of sunflower plants supplied with various amounts of boron, May 20, 1936, 47 days from planting of seed. (Photograph by Dr. J. W. MITCHELL.)

As shown by figures 3 and 4 and table II, boron at a concentration of 0.1 p.p.m., caused a very great increase in the height and weight of the plants. But the greatest growth was in the case of the plants supplied with boron at a concentration of 1.0 p.p.m. This was true in each experiment, though in 1935 the roots of the 0.25 p.p.m. weighed the most. Yet as is mentioned later, 1.00 p.p.m. was definitely toxic. The optimal concentration should, therefore, be regarded as lying between 0.5 p.p.m. and 1.0 p.p.m. The decrease in the weight of the plants in the 0.5 p.p.m. series of the 1935 experiment should not be regarded as significant but attributed to individual variation of plants. The number of plants was rather few.

The growing period of the 1935 experiment was a few days longer than the 1936 experiment; yet, as shown by table II, the 1936 plants made much better growth than the 1935 plants. This is to be expected since the light was better and the days longer in the spring than in the autumn. But because of this greater growth, it would also be expected that boron deficiency would develop sooner, would be more pronounced, and would have a greater effect on the weight of the plants in the spring than in the autumn. The reverse was true. As mentioned later, boron deficiency did not develop as soon in the spring as in the autumn, and table II shows that leaving boron out of the nutrient solution did not have as great an effect on the weight of the plants as compared with the plus-boron series in the spring as in the autumn. Figure 4 shows that the minus-boron plants grew to a considerable height. This no doubt indicates some boron contamination in the

spring experiment at least in the minus-boron series. As stated below, further work seemed to prove that this was true.

Definite symptoms of boron deficiency developed in several plants of the 1935 experiment in two weeks, while in the spring of 1936 no definite symptoms were noticed until 25 days after planting, and then only one or two plants showed the effects. To test out the hypothesis that this later development of the symptoms in the spring than in the autumn indicated some boron contamination at least in the minus-boron series, a few more pots of carefully washed sand were planted with sunflower seed on May 1, 1936. By May 20, definite symptoms of boron deficiency had developed in the minus-boron plants. It is possible that the symptoms were present a few days before this date.

The main symptoms of boron deficiency in the sunflower are as follows. The young leaves are lighter in color. Often they appear silvery white, especially in the lower half, and they may also have a bronze tinge. The lighter color frequently takes the form of a mottling of the leaves, though this is probably more characteristic of older stages of the deficiency. The young leaves often bend downwards against the stem. The crinkled character of the leaves and the curling under of the tips and edges are prominent characters. Sometimes the upper leaves are considerably dissected or lobed. Figures 5 and 6 show some of these characteristics. The plus-boron leaves are not quite normal because of a slight wilting.

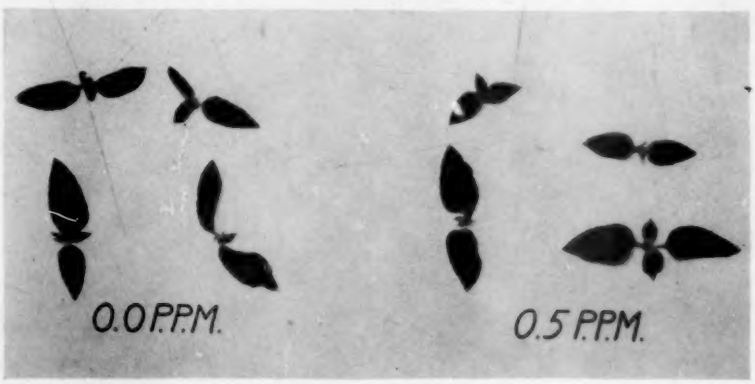

FIG. 5. Young leaves of plus- and minus-boron sunflower plants, May 25, 1936, 24 days from planting of seed.

Though we did not determine the optimum concentration for the growth of the soy bean, it is clear from this work that the sunflower requires more boron than does the soy bean. In growing sunflowers it is best to have boron at a concentration of about 0.5 p.p.m. constantly in the nutrient solu-

tion, while soy beans do well with an application twice a week at a concentration of 0.25 to 0.5 p.p.m.

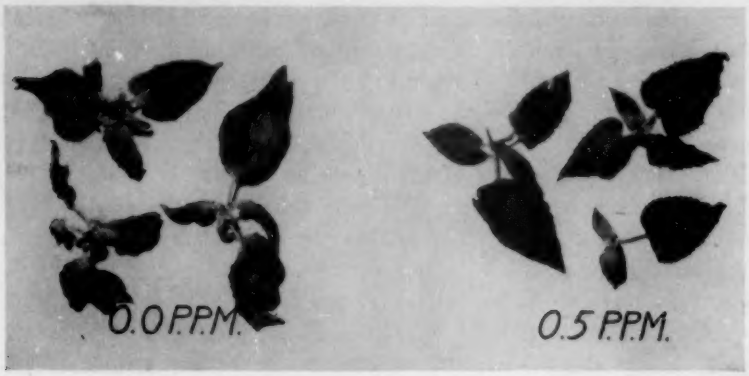

FIG. 6. Young leaves of plus- and minus-boron sunflower plants, May 21, 1936, 48 days from planting of seed.

BORON TOXICITY

SOY BEAN.—No work was done on the soy bean to determine at what concentration boron has a toxic effect. It might be expected, however, that a plant which needs little boron for optimal growth as compared with some plants, for example, the sunflower, would be very sensitive to an excess of this element. According to some of the literature this is true. COLLINGS (4) found that a concentration of 0.1 to 0.2 p.p.m. in the nutrient solution produced definite symptoms of toxicity on the leaves of the soy bean. It is interesting to note, however, that though this very low concentration was definitely toxic there was increased growth, though not a regular increase, up to a concentration of 2.5 p.p.m.

SUNFLOWER.—The minimum concentration of boron at which toxicity developed in this experiment was 1.0 p.p.m. The symptoms were definite at this concentration, though not so very pronounced. As mentioned earlier this is also the concentration giving the greatest growth. As the concentration was increased above 1.0 p.p.m., toxicity, as indicated by the appearance of the leaves, increased and the weights decreased. As shown by table II, this decrease was quite regular in the 1935 experiment, but the plants of the 15.0 p.p.m. series of the 1936 experiment weighed more than those of the 10.0 p.p.m. This is no doubt to be accounted for by individual plant variation. The number of plants was rather small.

Boron toxicity in the sunflower is indicated first by a mottling of the tips and edges of the lowest leaves. Later these mottled areas die. In severe cases of injury practically the entire leaf may be affected. Figure 7 gives some idea of the appearance of the leaves. The uninjured leaves do not

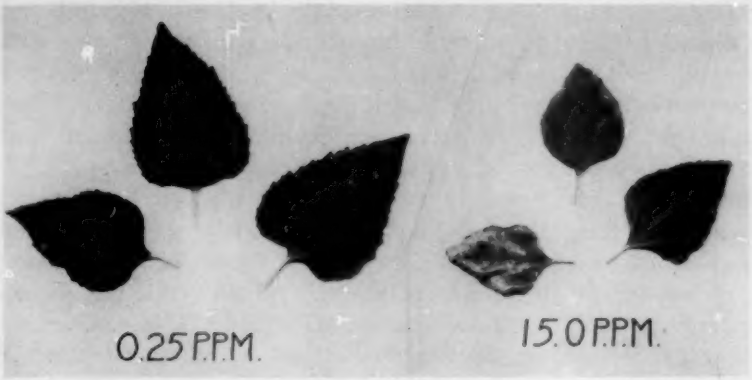

FIG. 7. Mature leaves of sunflower plants, showing toxicity of boron at a concentration of 15 p.p.m.

appear quite normal, because they had wilted somewhat before the photograph could be taken. The minimal time required for the toxicity symptoms to develop was not determined, but in the case of the 1936 experiment definite symptoms appeared in 3 weeks.

Discussion

BORON DEFICIENCY

The symptoms of boron deficiency are quite definite and characteristic, and once observed are easily detected. It is an element that affects meristematic tissue. The stem tip dies, and the growth of the root tip is also affected, death often resulting. In addition the young leaves become chlorotic, and malformations of various kinds may develop. The effects are similar for different plants, especially as regards the effects on the plant's apices, but differ in detail. For example, in this work the chlorosis of the young soy bean leaves gave them a streaked appearance, the interveinal tissue being affected, though white spots also developed; in the case of the sunflower, the young leaves became silvery white, especially in the lower half, and later there was a mottling of the leaves. The two plants are similar in the dying of the stem tips. These symptoms correspond well with those reported by other investigators (**3, 8, 11, 12**).

Plants, of course, differ decidedly as to the optimal concentration of boron for growth. If one defines optimal concentration as that concentration causing the greatest growth without any indications of toxicity, then the optimal concentration for growth of the sunflower lies between 0.5 and 1.0 p.p.m. The greatest growth was at 1.0 p.p.m., but there were also definite toxicity symptoms at this concentration. The effects of different concentrations were not studied in the case of the soy bean, but since deficiency

symptoms showed up much later in the soy bean than in the sunflower, and since normal soy bean plants were grown by an application twice a week of boron at a concentration of 0.25 to 0.5 p.p.m., while for best growth of the sunflower boron at a concentration of about 0.5 p.p.m. was kept constantly in the nutrient solution, it would seem that the optimal concentration for the growth of the soy bean is less than that for the sunflower.

These results compare fairly well with the results of other investigators. SOMMER and LIPMAN (**17**) used 0.5 p.p.m. of boron for their controls in their study of the effects of boron deficiency on the growth of the sunflower and other plants, though they did not determine whether this was the optimal concentration. MCHARGUE and CALFEE (**11**) found 0.7 p.p.m. optimal for the growth of lettuce, and SWANBACK (**18**) 0.4 p.p.m. for tobacco. On the other hand, COLLINGS (**4**) found that 0.1 to 0.2 p.p.m. was toxic to the soy bean, although there was stimulation of growth up to a concentration of 2.5 p.p.m. This would seem to support our conclusion given above that the soy bean requires less boron than the sunflower. BRENCHLEY and WARINGTON (**3**) showed that boron is essential for the normal growth of the soy bean, scarlet runner bean, crimson, red, yellow, and wild white clover, and the broad bean. On the other hand, they found that the pea, barley, and candy tuft could complete their entire life cycle, including flower and fruit development, without any boron in the nutrient solution, as shown by spectroscopic examination. It is admitted, however, that the two groups may differ merely in the amount of boron required, that if still lower amounts of boron could be achieved the second group might merge into the first. The seed of the plants of the latter group may contain enough boron for their complete development. Thus these results do not exclude the possibility that boron may be needed by all higher plants.

The conditions under which the plant is grown affects its sensitiveness to lack of boron. The Manchu soy bean did not develop symptoms of boron deficiency as soon when grown in the autumn as when grown in the spring. Similarly, WARINGTON (**20**) found in the case of various plants that the deficiency symptoms were less pronounced and their progress retarded under a short day as compared with a long day. MCMURTREY (**12**) found that aerated cultures of tobacco grew more vigorously and developed more pronounced symptoms of boron deficiency than unaerated.

BORON TOXICITY

While boron deficiency symptoms develop first on the youngest leaves of the plant, boron toxicity shows up first on the mature leaves at the base of the plant. The main symptoms as observed in the sunflower are a mottling of the tips and edges of the lower leaves, later followed by the death of this tissue. In severe cases practically the entire leaf is affected. These

symptoms are similar to those observed by other investigators (**5, 11, 16, 19**).

Plants of course vary decidedly as to their susceptibility to boron excess. In the case of the sunflower, toxicity was first observed at a concentration of 1.0 p.p.m., though if a closer series had been run, this critical toxic concentration might have been lowered somewhat. There was, however, no evidence of toxicity at 0.5 p.p.m. McHargue and Calfee (**11**) found that 0.9 p.p.m. of boron was the lowest concentration toxic to lettuce. On the other hand, the soy bean appears to be injured by a much lower concentration of boron than either the sunflower or lettuce. We did not determine the critical toxic concentration for the soy bean, but since our work indicates that the soy bean requires less boron for optimal growth than does the sunflower, it would be expected that toxicity would show up at a lower concentration. The work of Collings (**4**) indicates that this is true. He found that 0.1 to 0.2 was definitely toxic to the soy bean. Similarly, Warington's (**19**) results showed that boron was much more toxic to some plants than to others. For example, boron at a concentration of about 0.06 p.p.m. was toxic to barley, while in the case of the broad bean it took a concentration of about 1.6 p.p.m. or higher for toxic effects to show up. Eaton (**5**) classifies a number of crops into three groups as regards their susceptibility to boron excess: sensitive, semitolerant, and tolerant.

The conditions under which the plant is grown affect the toxicity of boron. For example, Brenchley's (**1**) results showed that plants can stand a higher concentration of toxins in the summer than in the early spring and the late autumn, and Warington (**19**) found that boron was not as toxic to the broadbean when it was grown in the summer as when it was grown in the early autumn or spring, owing perhaps to the more vigorous growth in the summer. In the case of boron deficiency, as pointed out earlier, the more vigorous the growth, the more pronounced are the deficiency symptoms, owing no doubt to the greater need of boron.

As previously stated, the greatest growth may be at concentrations that are clearly toxic as shown by the effects on the leaves. A concentration of boron of 1.0 p.p.m. was definitely toxic to the sunflower; the plants of this series, however, were decidedly larger than those grown at a concentration of 0.5 p.p.m. which was not toxic. In the case of the soy bean, Collings (**4**) found that though 0.1 to 0.2 p.p.m. was definitely toxic there was increased growth though not a regular increase up to 2.5 p.p.m.

The range between toxicity and deficiency of boron is quite narrow. At a concentration of 0.5 p.p.m. there was good growth of the sunflower, but 1.0 p.p.m. proved definitely toxic; as mentioned above, the latter concentration afforded better growth. McHargue and Calfee (**11**) found that 0.7 p.p.m. was optimal for the growth of lettuce and that 0.9 p.p.m. was slightly toxic and 1.2 p.p.m. decidedly so.

Summary

1. The main symptoms of boron deficiency in the sunflower and the soy bean are: a dying of the stem tip, a chlorosis of the young leaves, and certain malformations of these leaves. The main symptom of boron toxicity in the sunflower is a mottling of the older leaves, especially at the tips and edges, followed later by the death of this tissue.

2. Boron deficiency affects the youngest leaves of the plant first; boron toxicity, the oldest. This is well illustrated by the sunflower.

3. Plants differ decidedly as to their susceptibility to boron deficiency and excess. The sunflower requires a rather large amount of boron for optimal growth and is not injured until the concentration becomes quite high; on the other hand, the soy bean requires a rather small amount of boron, but, according to COLLINGS (**4**) is injured by very low concentrations of this element.

4. The conditions under which the plant is grown affects its susceptibility to boron deficiency and excess. The more vigorous the growth the more pronounced are the boron deficiency symptoms, but the less pronounced are the boron toxicity symptoms. The soy bean did not develop boron deficiency so soon when grown in autumn as when grown in the summer; on the other hand, the work of others has shown greater toxicity of boron when the plant is grown in the autumn or spring than when grown in the summer.

5. There may be better growth at concentrations of boron that are definitely toxic. Boron at a concentration of 1.0 p.p.m. was toxic to the sunflower, but the plants of this series were larger than those supplied with less boron.

6. The range between boron deficiency and toxicity is quite narrow. Boron at a concentration of 0.5 p.p.m. afforded good growth of the sunflower, but 1.0 p.p.m. was definitely toxic.

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LIGHT-MASS ABSORPTION DURING PHOTOSYNTHESIS

EARLE AUGUSTUS SPESSARD

(WITH FIVE FIGURES)

Introduction

In this paper, and in one to follow it, data will be presented to show that light-mass absorption occurs during photosynthesis. The present paper submits the experimental evidence obtained relative to error, to surface phenomena due to atmospheric conditions during weighing operations, and to the question of CO_2-leakage through glass containers as possible explanations of the weight data.

Experimentation

General plan of the experiment

LIGHT-ABSORBER.—Plants capable of photosynthesis, and at the same time capable of withstanding the conditions inside an hermetically sealed container, had to be chosen. A preliminary series of extermination tests involving some 5000 mixed cultures of green and blue-green algae were run between 1917 and 1934. Invariably the blue-greens survived the greens. No great difference, however, was observed between the blue-greens and the unicellular greens. These forms were, therefore, chosen as the light-absorbing material.

PROVISION FOR CO_2.—At the time of inoculation and sealing, the plants were in a balanced aquarium condition with protozoa. The source of CO_2 supply for photosynthesis was, therefore, the respiration of both plants and animals, and the decay of dead organisms. This eliminated the necessity for CO_2-leakage through the walls. If, however, the animals should not survive the period of experimentation the necessity for CO_2-leakage would arise. Accordingly a preliminary test to determine this point was made from 1934 to 1935. Samples of a culture containing both plants and animals were sealed within glass tubes. These were examined at the end of 1935, microscopically, through the glass. It was observed that some flagellates were active at the end of that time. While we know, therefore, that the internal supply of CO_2 was present we do not know whether it was adequate.

PROVISION FOR TESTING CO_2-LEAKAGE.—The adequacy of the CO_2 supply not being established, it was, therefore, necessary to determine whether the gas could leak through the walls of the container during the course of the experiment. Solutions of $Ba(OH)_2$ and of KOH were sealed in glass tubes in 1934. At the end of 1935 there appeared to be some indication, precipitates, that CO_2 had leaked through the glass, or that the glass had been attacked with the production of precipitated silicates. In such a case, if

CO_2 diffuses through, so would O_2 and N_2, which would cause weight changes. Additional tests were made, as reported in the following pages.

PROVISION FOR TESTING CO_2-LEAKAGE THROUGH THE WALLS OF THE PLANT FLASK.—CO_2-leakage through the walls of flasks containing KOH might be ascribed to deteriorated walls rather than to defective walls. It would not constitute proof that the plants received their CO_2 supply from the outside. In the case of KOH, the partial vaccuum for that gas would be very high, while in the case of the plant flask, very low, owing to the internal supply. It was decided, however, to run a test in the following manner. During the first half of the experiment one plant flask was kept in normal air like all other flasks. During the second half the same plant flask was placed in CO_2-free air. The flask was placed in a 2-liter desiccator containing 100 ml. of saturated KOH. If the internal supply of CO_2 were inadequate for the requirements of photosynthesis, then the plant flask should gain in weight during the first half, provided leakage occurred, while during the second half, it should not gain in weight.

PROVISION FOR DETERMINING THE EFFECT OF BUOYANCY, TEMPERATURE, AND MOISTURE.—The flasks to be weighed were matched, as nearly as possible, with another in which water was placed, so that the counterbalance weights required would be as low as possible. Since it was impossible to determine accurately the difference in volumes on the two pans, a preliminary series of weighings was made with flasks and with beakers, the volume of one being approximately twice that of the other. These tests showed that the weights varied directly as the temperature when the greater volume was on the left pan. Factors could be worked out, therefore, and corrections for buoyancy be determined, provided variations in atmospheric conditions required their use, and provided also, that it could be determined how the weights followed the temperature. It was decided to avoid factors entirely by weighing the flasks under the same atmospheric conditions during at least the start and finish of the experiment.

PROVISIONS FOR REDUCING ERROR DURING WEIGHING.—It was desired to weigh accurately to within 0.02 mg. The balance, an Ainsworth, Type DLM, had a sensitivity, using the single deflection method, of 5.6. This means that it was possible to secure a delivery of less than 0.02 mg. The rider-width, beam marks, and spaces were all measured and plotted on paper. By this means it was possible to determine seating positions very close to 0.01 mg. An arbitrary rest point of + 6.8 was used at the start and finish. The readings in between were taken at + 5.0. Since, however, the change of rest point is significant when using double deflection, both points were used at the last set of readings. No significant difference was observed. By selecting a constant reading point, there was no difficulty in observing a change of deflection on the reseating of the rider 0.01 mg.

To take readings consistently at this value, time intervals had to be established for various manipulation steps, as follows: a, beam release, 5 min.; b, pan release, 10 min.; c, opening balance, 30 min.; d, washing flasks, 6 hr., and 24 hr. for check; e, brushing flasks, 30 min.; f, rest period for eye, while closed, 2 min. Failure to observe these points results in an error ranging from 0.02 to 0.1 mg.

The rest point was determined both before and after weighing the flasks. If it changed during the weighing, the whole was repeated.

Under certain atmospheric conditions, the sensitivity of the balance becomes suddenly reduced, and may be lost entirely. It is apparently an electrical phenomenon. Its duration may be either one hour or ten days. Its explanation needs investigation. A slight effect is not observable when using double deflection, but under single deflection, the behavior is revealed at once, by the refusal of the return deflection to be complete. Weighing accurately to 0.01 mg. is impossible when this singular behavior is at all apparent. It is partially connected with relative humidity. When that is 64 per cent., the effect is somtimes predictable, but not always.

PREPARATION OF FLASKS AND ARRANGEMENT FOR WEIGHING.—Five K-exax flasks of 25-ml. capacity were selected from the same shipment, cleaned and prepared as follows:

Flask no. 1, plant flask, in which were placed 5 ml. of mud on which blue-green algae were growing with various protozoa.

Flask no. 2, water flask, with 15 ml. sterile distilled water.

Flask no. 3, KOH flask, with 15 ml. N/5 KOH.

Flask no. 4, KOH flask, with 15 ml. N/10 KOH.

Flask no. 5, water flask, with 15 ml. sterile distilled water.

These five flasks were arranged in pairs, so that the plant flask would be weighed against both the N/5 KOH and water flask no. 2, and the KOH flask would likewise be weighed against water flask no. 2, as follows:

Pair 20, left pan, water flask no. 2; right pan, plant flask and weights.

Pair 22, left pan, water flask no. 2; right pan, N/5 KOH flask and weights.

Pair 23, left pan, N/5 KOH flask; right pan, plant flask and weights.

Pair 21, left pan, N/10 KOH flask; right pan, water flask no. 5, and weights. (This pair was considered a check for pair 22.)

The procedure was to weigh the three pairs 20, 22, and 23 during the same weighing period, pair 21 being carried through separately.

In the set-up, 20, 22, and 23 the object is, in each case, on the right pan with the weights. If the object gains in weight, this will be indicated by a loss in the value of the counterbalance weights. The reverse is true in pair 21.

In pairs 20, 22, and 23 the recorded gains in weight will form a cross check as follows: let the plant flask in pair 20 gain n mg. Let the KOH

flask in pair 22 gain w mg. Then the plant flask in pair 23 will gain $n-w$ mg. If the reading error is 0.01 mg., the three pairs should check within 0.03 mg., the standard deviation being 0.005 mg.

In addition to the four pairs mentioned, 10 others were prepared to determine the correlation between observed weight-gain and photosynthetic activity. These will be described in full in a later paper.

Weighing data for pairs 20, 22, 23, and for pair 21

The weighing data are given complete as recorded, in tables I, II, III, and IV. The standard deviation, calculated in the usual manner, is given for each period. The values plotted in figures 1, 2, 3, and 4 are the means by periods. No corrections are necessary either for buoyancy or for temperature. The weights follow the temperature slightly, and inversely. A correction factor for temperature having a value of 0.0025 mg. per degree of temperature change, and a buoyancy correction for 1 ml. excess volume on the right pan, in each case, does not significantly alter the results obtained before correction. Since we desire only the beginning and end readings, the atmospheric conditions are so nearly alike that the effect of the difference is less than the reading error.

TABLE I

Weighing data of pair 20.* Left pan, H_2O flask; right pan, plant flask and 13.622 gm. counterbalance weights

Period	Date	Temperature of balance	Pressure in mm. Hg.	Apparent weight	Deviation from mean	Standard deviation
	1938	°C.	mm.	mg.	mg.	mg.
I	2/22	18.4	771	2.45	0.012	
	23	20.6	770	2.44	0.002	
	3/ 4	24.3	755	2.41	0.028	
	6	21.3	773	2.44	0.002	
	7	19.7	777	2.45	0.012	0.01
Means:	3/ 1	20.9	769	2.438		
II	3/31	22.3	765	2.40	0.003	
	4/ 4	19.8	759	2.40	0.003	
	11	18.6	767	2.40	0.003	
	12	18.2	768	2.41	0.007	0.005
Means:	4/ 6	19.7	765	2.403		
III	6/ 3	26.7	765	2.35	0.00	
	8	26.0	764	2.35	0.00	0.00
Means:	6/ 5	26.3	765	2.35		
IV	10/28	21.0	766	2.29	0.00	
	29	21.8	768	2.29	0.00	0.00
Means:	10/29	21.4	767	2.29		

* Note: Plant flask was in CO_2-free atmosphere from 6/8 to 10/28.
Mean time: 243 days.
Gain in weight by plant flask: 0.148 mg.
Annual rate of gain: 0.219 mg.

TABLE II

WEIGHING DATA OF PAIR 23.* LEFT PAN, KOH FLASK; RIGHT, PLANT FLASK AND 13.499 GM. COUNTERBALANCE WEIGHTS

PERIOD	DATE	TEMPERATURE OF BALANCE	PRESSURE IN MM. HG.	APPARENT WEIGHT	DEVIATION FROM MEAN	STANDARD DEVIATION
	1938	°C.	mm.	mg.	mg.	mg.
I	2/22	18.6	771	9.50	0.004	
	23	20.8	770	9.50	0.004	
	3/ 4	20.6	771	9.50	0.004	
	4	24.5	755	9.48	0.016	
	5	24.2	757	9.50	0.004	
	7	20.3	774	9.49	0.006	0.008
Means:	3/ 1	21.5	767	9.496		
II	4/ 9	17.9	766	9.47	0.002	
	9	19.2	767	9.47	0.002	
	10	18.0	770	9.48	0.008	
	11	18.5	766	9.47	0.002	0.005
Means:	4/10	18.4	767	9.472		
III	6/ 3	26.7	765	9.45	0.004	
	3	26.5	765	9.45	0.004	
	7	27.2	760	9.44	0.006	0.005
Means:	6/ 5	26.8	763	9.446		
IV	10/28	23.0	766	9.42	0.00	
	29	20.5	766	9.42	0.00	
	29	21.8	767	9.42	0.00	
	330	19.8	769	9.42	0.00	0.00
Means:	10/29	21.5	767	9.42		

* Note: Plant flask was in CO_2-free atmosphere from 6/8 to 10/28.
Mean time: 243 days.
Gain in weight by plant flask: 0.076 mg.
Annual rate of gain: 0.113 mg.

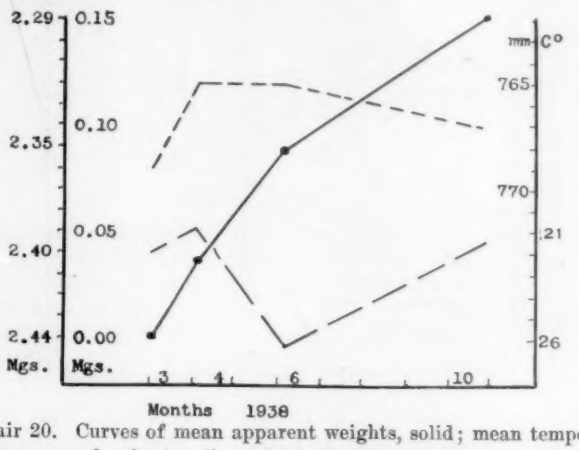

FIG. 1. Pair 20. Curves of mean apparent weights, solid; mean temperature, coarse broken; mean pressure, fine broken lines. All plotted against mean time period. Right milligram column, gain in weight made by the plant flask; left milligram column, values given in table I.

TABLE III

WEIGHING DATA OF PAIR 22.* LEFT PAN, H_2O FLASK; RIGHT PAN, KOH FLASK AND COUNTERBALANCE WEIGHTS, 0.122 GM.

PERIOD	DATE	TEMPERATURE OF BALANCE	PRESSURE IN MM. HG.	APPARENT WEIGHT	DEVIATION FROM MEAN	STANDARD DEVIATION
	1938	°C.	mm.	mg.	mg.	mg.
I	2/23	20.7	760	2.80	0.00	
	24	20.2	772	2.80	0.00	
	3/ 5	24.0	766	2.80	0.00	
	6	21.5	773	2.80	0.00	0.00
Means:	3/ 1	21.6	767	2.80		
II	3/29	20.0	759	2.80	0.002	
	31	22.0	765	2.80	0.002	
	4/ 4	20.0	759	2.78	0.018	
	9	19.1	765	2.80	0.002	0.009
Means:	4/ 3	20.3	762	2.798		
III	6/ 3	26.2	767	2.77	0.000	
	3	26.2	764	2.78	0.01	
	5	27.0	768	2.78	0.01	
	8	26.0	767	2.77	0.00	
	8	26.3	765	2.76	0.02	
	21	27.3	766	2.76	0.02	0.013
Means:	6/11	26.5	766	2.77		
IV	10/28	23.0	766	2.74	0.02	
	29	21.0	768	2.70	0.02	
	29	22.0	767	2.72	0.00	0.015
Means:	10/29	22.0	767	2.72		

* Mean time: 243 days.
Gain in weight by KOH flask: 0.08 mg.

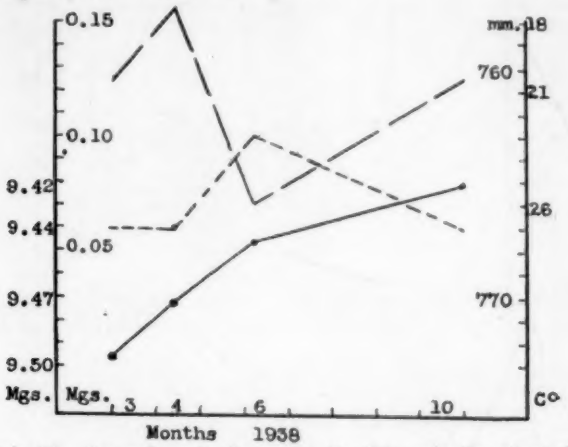

FIG. 2. Pair 23. Legend as in figure 1. In this pair the plant flask of pair 20 (fig. 1) is shown as weighed against N/5 KOH, pair 22 (fig. 3). The plant flask here gained 0.076 mg., while in pair 20, it gained 0.148 mg. This is interpreted as meaning that the KOH flask absorbed CO_2 in sufficient amount to account for the difference of 0.072 mg. The actual gain in weight as shown in pair 22 for the KOH was 0.08 mg. The agreement is very close.

TABLE IV

WEIGHING DATA OF PAIR 21.* N/10 KOH WEIGHED AGAINST WATER. LEFT PAN, KOH; RIGHT PAN, WATER FLASK AND 0.974 GM. COUNTERBALANCE WEIGHTS

PERIOD	DATE	TEMPERATURE OF BALANCE	PRESSURE IN MM. HG.	APPARENT WEIGHT	DEVIATION FROM MEAN	STANDARD DEVIATION
	1938	°C.	mm.	mg.	mg.	mg.
I	2/22	21.0	771	4.52	0.00	
	23	21.0	769	4.52	0.00	
	24	24.0	766	4.52	0.00	0.00
Means:	2/23	22.0	768	4.52		
II	3/ 5	22.0	769	4.52	0.00	
	6	20.2	774	4.52	0.00	0.00
Means:	3/ 6	21.1	771	4.52		
III	6/22	27.9	766	4.55	0.006	
	23	27.3	765	4.56	0.004	
	24	28.0	766	4.56	0.004	0.005
Means:	6/23	27.7	766	4.556		
IV	10/25	21.8	766	4.60	Single reading	

* Mean time: 243 days.
Gain in weight by N/10 KOH flask: 0.08 mg.

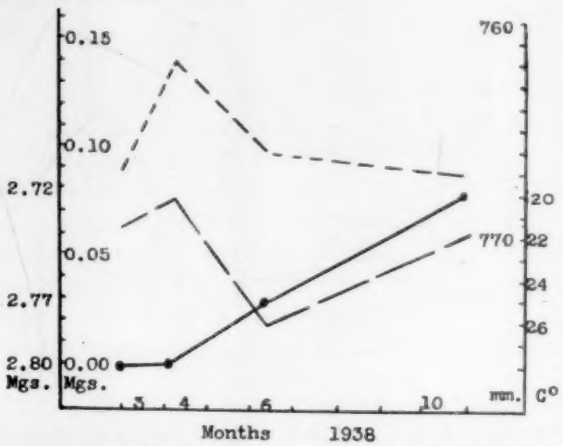

FIG. 3. Pair 22. Legend as in figure 1. The N/5 KOH flask gained 0.08 mg., apparently due to leakage of CO_2 or other gases following deterioration of the glass.

ANALYSIS OF WEIGHING DATA

Without any corrections, the weights for all four points in pairs 20, 22, and 23 check within 0.012 mg. After correcting 20 and 23 as stated above, none being required for pair 22, the weights check within 0.014 mg. This is well within the limits of reading error of 0.03 mg. set previously, in so far as the endpoints, I and IV alone are concerned.

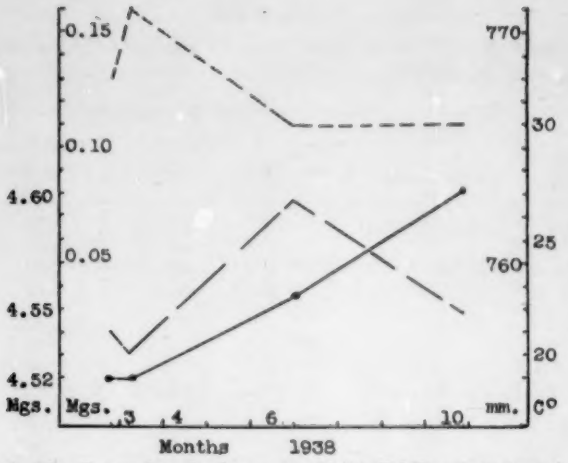

FIG. 4. Pair 21. Legend as in figure 1. N/10 KOH weighed against water. The record is essentially similar to that of the N/5 KOH flask, pair 22, figure 3.

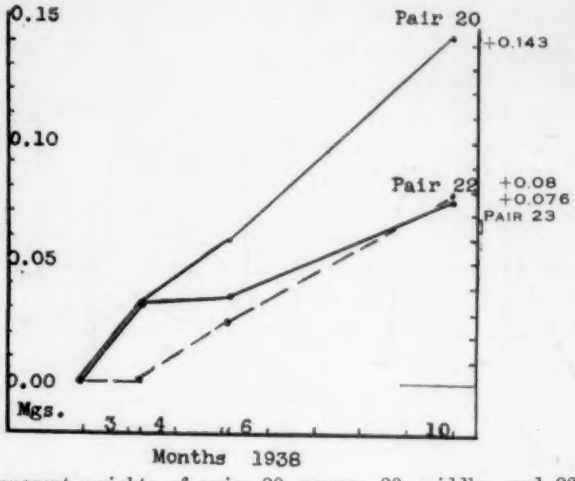

FIG. 5. Apparent weights of pairs 20, upper; 23, middle; and 22, lower, after correcting for buoyancy of 1 ml. for 20, 0.5 ml. for 23, and 0.02 ml. for 22. All points in agreement within 0.03 mg.

Point III is also a critical point when we come to consider whether the rate of weight gain remained the same after June, when the plant flask was placed in CO_2-free atmosphere. A glance at figures 1, 2, and 3 indicates that a slight break occurred in the weight curve of all three pairs at the temperature peak. Correcting for buoyancy alone, figure 5, no doubt partly corrects for temperature also. It is interesting to note that a correction, $1° = 0.005$ mg., for pair 20, yields almost the duplicate of the curve

representing 1 ml. buoyancy correction shown in figure 5. For pair 23, the buoyancy correction curve of 0.5 ml. duplicates the temperature curve of $1° = 0.0025$ mg.

The precise differences in volume between the two pans could in no case be determined accurately. To correct for buoyancy it is at least necessary to know on which pan is the greater volume. A number of tests were made to determine this point. In all three pairs, 20, 22 and 23 the volume on the right pan is slightly greater than on the left. Furthermore, this difference is greatest in pair 20, less in pair 23, and least in pair 22. By immersing the flasks of pair 20 in water and calculating the volume of the weights, it was determined that the difference in volume for this pair is approximately 1 ml. From the relative behavior of the two pairs, 20 and 23, during the tests, the difference in volume for pair 23 was taken as about half that of pair 20. That of pair 22 was taken as less than half the latter. Corrections for buoyancy on this determination, yield curves for pairs 20 and 23 consistent with those uncorrected. In figure 5, such curves show that the rate of gain after June was, within error, the same as before.

It appears then, from the data, that the gain in weight observed for the plant flask and for the KOH flasks, is not attributable either to error in weighing or to surface phenomena.

Though the data are not precise on the slight corrections required, after the effect of buoyancy is allowed, it appears probable that the plant flask gained in weight, after transfer to CO_2-free air, at the same rate as before. It should be pointed out that a change in the vitality of the plant after transfer, caused either by a change in light intensity or by increased temperature during the hot summer, might conceivably cause a lowering of the rate of gain. Actually, then, the curve of weights without correction may be as near the true situation as those after correction. In any experiment of this kind, the vital history of the plant is an essential factor. This point will be demonstrated significantly in a succeeding paper.

There remains for consideration, the question of CO_2-leakage through the walls of the plant flask. The fact that the gas has possibly been demonstrated to have leaked through the walls of the KOH flasks is not proof that it leaked through the walls of the plant flask. For in the case of the KOH flask, it is well known that KOH attacks glass. Leakage in those flasks was therefore probably made possible by deteriorated walls, rather than by defective ones. Fortunately, this was anticipated as already stated, by placing the plant flask in CO_2-free air during the last half of the observation period.

From February to April the readings as shown in tables I, II and III, indicate that leakage of CO_2 did not occur, the weights of the KOH flasks remaining the same. During that same period, the weights of the plant flask did show a gain. This is evidence that the gain in weight is not at-

tributable to leakage of CO_2. The proof of this inference lies, however, in the subsequent history of pair 20. From June to Oct., the external supply of CO_2 was cut off from the plant flask. Nevertheless the plant flask continued to gain in weight at approximately the same rate as when the external supply was available. There is therefore no apparent reason for assuming that CO_2 diffused into the plant flask at any time during the experiment.

This suggests another possible source of error. Assume that any of the gases included within the flask at the time of sealing subsequently diffused in part outwardly. A loss of gas would have caused a loss in weight. But, due to its compressibility, the volume of the flask may have therefore been reduced. A reduction in the volume of the plant flask, while the volume of the water flask remained constant, would decrease the buoyancy of the plant flask causing an apparent increase in weight. It would require only a decrease of 0.2 ml. volume to account for the observed annual rate of weight gain, namely, 0.22 mg. This is answered by the fact that the weight of the escaped gas would approximately cancel the loss in buoyancy, assuming for the moment that the flask would be capable of collapsing the amount required.

If, however, the internal pressure decreased gradually without a loss of gas, and if the flexibility of the glass permitted a decrease of one per cent. of the volume of the flask, then the change in buoyancy would account nicely for the observed increase in weight by the plant flask.

Consider first the property of the glass. If we assume 25×10^{-6} as the coefficient of cubic expansion for glass, it would require a lowering of the internal pressure one atmosphere to reduce the volume of the glass 0.0006 ml. This figure is obviously too low to account for the observed increase in weight.

Consider next the volume change in the flask due to the fact that glass is flexible. As a rubber balloon may be inflated or deflated by varying the internal gas pressure, so may we assume changes in a sealed glass flask. So far I have not been able to secure any tables or other data on the degree of volume changes in bulbs or sealed glass vessels. It is obvious that such objects must change in volume to a small extent with variations in internal pressure. But I am assuming that such changes are much less than one per cent. of volume, the amount required to account for the observed increase in weight reported here.

We come finally to the probability of a decrease in the internal volume of gas. Since the immediate question is being considered independently of the question of leakage, the rare gases are ruled out because they are inert. Assume for the moment that for some reason they all leaked out of the flask, even though the percentage of argon exceeds that of CO_2, the total loss of

the rare gases would account for 0.02 ml., assuming further that the glass was flexible enough to take up that volume, and that the decrease in pressure permitted such a change in volume.

At the time of sealing, there was not more than 0.006 ml. of CO_2 in the flask. A thorough depletion of this gas by changing it to solid or liquid compounds within the flask, would therefore not account for the required volume change of 0.2 ml.

The case for O_2 is somewhat different. The excess of O_2 resulting from the process of photosynthesis would be taken up by CO_2 consumption. The respiration of the plants, however, might have given a respiratory quotient less than unity due to the fact that the food storage products are largely fats. If so, there would have resulted an excess pressure of O_2, resulting in an increase in flask volume, resulting in turn in a decrease in weight due to a change in buoyancy under the conditions mentioned earlier. If we consider that animals enclosed at the time of sealing, depleted this excess of O_2 even below the normal volume, such depletion is balanced by the CO_2 given off by the animals during their respiration.

There remains for consideration the gas, nitrogen. It is possible to assume that nitrogen fixing bacteria were included in the culture at the time of sealing. They would take N out of the gaseous state into a solid state, thereby reducing the internal pressure. If complete, the internal gas volume would have been reduced approximately 16 ml. and the internal pressure lowered perhaps 0.7 atmosphere. Assuming that the glass was flexible enough to collapse one per cent. of its volume, this decrease in internal pressure would be sufficient to permit the change. But there is an objection to this interpretation. The plants in all probability used up the nitrogen compounds in their metabolic processes, restoring the N to the internal atmosphere.

It is impossible to decide without chemical analysis of the flask contents at the beginning and end of the experiment, whether the normal nitrogen cycle occured or not. Obviously such an analysis is an impossibility. The impossibility of determining a point in question does not validate data, of course, but rather does it emphasize incompleteness in the experimental arrangement. In other words, while the N question has been answered theoretically, the question lacks exact experimental confirmation.

Concluding remarks

It has been reported in this paper that green plants gain in weight while insulated from their material environment. In the second part which is to follow, data will be presented on 10 other cases, showing that such weight gain is to be correlated with the process of photosynthesis only. The inference will there be drawn to the effect that the gains in weight observed are

explainable only on the assumption that light is absorbed as mass during photosynthesis.

This brings us at once into conflict with Einstein's equation $E = M C^2$. This equation sets the mass limits obtainable through such an experiment as this. The values obtained in the experiment, are, according to various calculations given me, something like three decimal points too high. The equation itself has been verified experimentally from so many independent lines of approach that we are forced to seek an explanation of the discrepancy between the limits set and the values obtained in the present experiment. There are two alternatives. First, the present data are the result of some error or are explainable on some other grounds than the one suggested. Second, an unknown factor in light absorption during photosynthesis precludes a proper application of the equation to the problem.

It is our intention to reexplore the first alternative.

HENDRIX COLLEGE
CONWAY, ARKANSAS

DETERMINATION OF MINERAL ELEMENTS IN PLANT TISSUE[1]

Introduction

The response to the first supplement of methods for the chemical analysis of plant tissue has convinced the committee that such treatment of its function should be undertaken only at relatively long intervals. Only as major improvements in procedure arise is substantial service through this channel likely to be feasible. With the emphasis already placed upon the major groups of organic constituents, *viz.*, carbohydrates, nitrogen-containing compounds, and lipids (SANDO, **26**) there has seemed to remain most obviously for consideration the so-termed inorganic elements.

Current interest in the physiological functions of the rarer mineral elements certainly seems to justify consideration of the more pertinent members of the inorganic group. In view of the rapidly expanding information concerning the occurrence of elements of this group in proteins, and their highly essential rôles in the regulation of enzyme action, the artificiality of attempting to limit their functions by use of the term "inorganic" becomes apparent. The subject here treated is the common meeting ground of students in the fields of both nutrition and metabolism. In the matter of testing for deficiency of rarer mineral elements it is possible that the biological test (plant response) will prove to be of considerable importance in relation to some of the extremely low concentrations involved, as suggested by MCMURTREY (**23**). Nevertheless, it does not appear at present that this diagnostic method will generally supercede determination of the elements in question but rather that it may be expanded and confirmed by the latter.

In passing, it may be advantageous to scan the issue of development in chemical methods of analysis. Perusal of the files of the analytical edition of Industrial and Engineering Chemistry tends to convey the impression of impermanence of results in this field. The considerable sequence of papers concerning some types of determination may be recognized, however, as inspired in part by the desire for absolute accuracy and the individualism incident to this end. For the usual requirement of comparative data on biological samples it appears that extreme accuracy may be sacrificed in favor of other qualifications, such as simplicity and speed. These procedures which are reasonably accurate (variable within a range of 5 to 10 per cent.), suffice for numerous biochemical investigations. In view of the general accessibility of sources of information here cited it has seemed inadvisable to quote procedures in detail. A noteworthy development in the adoption of chemical methods by plant physiologists is the substantial inclusion of these in laboratory manuals (**21**).

[1] Prepared by the committee on chemical methods, Dr. W. E. TOTTINGHAM, chairman.

Methods for the most abundant elements

Procedures for determining these constituents as given in Methods of Analysis (**2**) appear in the main to be as acceptable as any thus far developed. It should be known that critical examination of such procedures, and some issues in process of development, are published periodically in the Journal of the Official Agricultural Chemists. In view of the small proportions of mineral elements to be found in many types of tissue, it is advantageous to conserve material by applying semi-micro technique. The advantage of this form of procedure has been recognized to an increasing extent by the A.O.A.C.

Aluminum

In agricultural practice it was formerly customary to derive the value of this constituent in plant tissue from the combined precipitate of phosphates of iron and aluminum (**2**, p. 122). Aluminum was formerly determined indirectly by use of the value found for iron, but this element is now dealt with directly. Both colorimetric and spectrographic procedures are available for this purpose, the former apparently being more sensitive. A microcolorimetric method is presented tentatively by the A.O.A.C. (**2**, p. 122). The colorimetric procedure of Lundell and Knowles (**22**) might prove adaptable to plant analysis. It is sensitive to less than 0.1 mg.

Calcium

The micro-procedure of the A.O.A.C. (**2**, p. 123) by way of the oxalate should be generally acceptable. Phillips, Smith, and Dearborn (**25**) have suggested that a modification be applied to the usual procedure for removing silica. This involves fusion of the ash with Na_2CO_3, followed by liberation and evaporative dehydration of silicic acid. Titration of the liberated oxalic acid with $KMnO_4$ appears to be generally satisfactory, although Ellis (**9**) has introduced cerium as the oxidant. In back titration of the excess ceric compound, he reports a sharp end point.

Chlorine

The volatility of this element from some of its compounds has been recognized (Fraps, **13**) and is avoided by preparatory impregnation of the sample with Na_2CO_3 before ignition. The solution recovered from the ash can then be treated with excess of $AgNO_3$ and back titrated with KCNS, as advocated by the A.O.A.C. (**2**, p. 131). Direct titration with $AgNO_3$ using K_2CrO_4 as indicator does not seem to be feasible here as it is in water analysis. Wilkens (**32**) has developed a sensitive method for determining chlorine iodimetrically.

Magnesium

The long-time standard procedure of determining this element in the filtrate from calcium, by precipitating with ammonium as the double phosphate to be weighed as magnesium pyrophosphate (**2**, p. 124), appears likely to remain the preferred method for some time. It is noteworthy that a colorimetric method is available for the determination of as little as 0.06 mg. of this element with an error of about 3 per cent. (**14, 16**). With the exception of use of the centrifuge to eliminate contamination by filter paper, the procedure is given fully in Chemical Abstracts **16**: 2342. 1922. Satisfactory use of a procedure involving precipitation of magnesium by 8-hydroxy quinoline is reported by the Department of Agricultural Chemistry, Purdue University.

Phosphorus

The standard procedure for determining this element in fertilizer analysis is hardly applicable to the small amounts present in many types of plant tissue. Difficulty in filtering the ammonium phosphomolybdate, which may exist in too finely divided state as well as in extremely small proportions, is minimized by micro-procedure (**2**, pp. 130–131.). Essentially organic matter is destroyed by ignition with magnesium nitrate, and silicic acid is removed by the usual dehydration treatment. The phosphorus is then converted to ammonium phosphomolybdate, from which molybdenum may be reduced for accurate colorimetric measurement by comparison with a standard phosphate solution. It may be noted that the blue color of molybdenum here utilized has been applied to several determinations which permit the intermediate formation of a phosphate. Analysts in animal biochemistry have preferred aminonaphtholsulphonic acid over hydroquinone as the reducing agent, owing to off-colors of oxidation products and slower action in case of the latter. Nevertheless, hydroquinone appears to be satisfactory for multiple determinations in plant analysis.

Potassium

The determination of this element gravimetrically by way of the chlorplatinate (**2**, pp. 125–130) seems to have been accorded a relatively permanent place in analytical procedures. In view of the considerable cost of the precipitant, it may be helpful to call attention to the possibility of determining potassium, either volumetrically or gravimetrically, as the cobaltinitrite. The volumetric phase of this procedure qualifies favorably as to both time and expense involved.

As formerly commonly conducted in the presence of acetic acid (**1**) this precipitation was attended by irregular results. Volk and Truog (**31**) have investigated the requirements of this method thoroughly and have found

that the maintenance of carefully defined conditions during precipitation leads to reliable results. Chilling in an ice water bath during precipitation, and further also during filtration, serves to control the composition of the precipitate. Apparently one of the chief requirements of this method is that of holding cobalt in the oxidized state and thus permitting titrimetric oxidation of the nitrite radicle. SIDERIS (**27**) sought to avoid variability in composition of the cobaltinitrite precipitate by determining the cobalt colorimetrically. His procedure for thus determining potassium appears to add a slight element of complication. Attention should also be directed to the colorimetric procedure of MORRIS and GERDEL (**24**) based upon the cobalt content of the cobaltinitrite precipitate. Owing to the possibility of speed and replication, these investigators considered the method to compare very favorably with the chlorplatinate method. WILCOX (**33**) substituted nitric acid for acetic acid in the original cobaltinitrite procedure and thus held the cobalt in oxidized form at room temperature.

The following results were obtained by a moderately experienced analyst for a member of this committee, using twelve different samples of dried potato tuber tissue. The gravimetric determination of potassium as the chlorplatinate was used as a standard of reference for evaluating the volumetric cobaltinitrite procedure of WILCOX. Eight determinations by the volumetric method gave lower results than the gravimetric method, while four determinations gave higher results by the former procedure. The departure ranged from −7.4 to 1.4 per cent. of the corresponding gravimetric determinations, with an average departure of −2.3 per cent. It may be observed that the results are rather favorable to the relatively simple modification introduced by WILCOX.

SILICON

Although this element may occur in some samples primarily through contamination by soil, it is universal practice to remove it from the digest of the tissue ash. This precaution avoids contamination of some precipitates, and precludes interference with the phosphorus determination. Silicon is isolated by the familar procedure of evaporative dehydration of silicic acid contained in the acid digest of mineral matter. If samples of large silicon content are evaporated repeatedly, it may not be necessary to heat to 110°–120° as formerly specified. The presence of soil particles would necessitate a preparatory alkaline fusion. While the resultant silica may be weighed after filtration and ignition, it is more selective procedure to determine the loss of weight when it is volatilized as the silico-fluoride after treatment wth HF.

SODIUM

Apparently reliable, practicable methods for determining this metal are in the process of development. Until recently the A.O.A.C. has consistently

proposed that the contents of sodium be derived indirectly from the weight of mixed chlorides of sodium and potassium. This indirect procedure seems to have been rarely adopted, presumably because of infrequent interest in the determination of the element in question.

The A.O.A.C. (**2**, pp. 126–127) has presented a tentative gravimetric method for sodium, based on the formation of sodium-magnesium-uranyl acetate. BLENKINSOP (**5**) removed iron by gentle ignition of the ferric phosphate, added excess of titanous chloride, and titrated with iron alum. He found the procedure accurate to 0.1 mg. of Na. For those desiring greater refinement, it might be possible to adapt the photoelectric microprocedure of HOFFMANN and OSGOOD (**19**). This method, based on stabilization of the yellow color of uranyl-zinc-sodium acetate against temperature changes by the addition of NH_4SCN, is reported as subject to an error of but 1 per cent.

SULPHUR

It is commonly known that the primary object in determining this element is conversion to SO_4 for precipitation as the very insoluble barium salt. The A.O.A.C. (**2**, p. 129) offers an option between fusing with a mixture of Na_2CO_3/Na_2O_2 and similar treatment with Mg $(NO_3)_2$, for the purpose of oxidation.

As in other determinations, the rôle of a proper blank determination on the reagents should be recognized. Should one not have access to the fundamental analytical procedures cited from the Methods of the A.O.A.C., these may generally be derived from standard treatises on quantitative analysis.

Determination of rarer mineral elements

As information accumulates, it appears possible that the list of elements which will eventually be included in this category may become relatively numerous. With the exception of iron, however, those which are now known to be deficient in soils to an extent that might be considered common, are but four in number. It was formerly supposed that spectrographic procedure provided the ultimate degree of accuracy for determining some of these elements. Doubtless the expense of apparatus for this physical method will prevent its use by many physiologists. Those who are in a position to secure assistance in this respect from departments of physics or chemistry may be considered fortunate. Nevertheless, as will appear presently, difficulties in adequacy of refinement inhere even in spectrographic equipment. Fortunately, the biochemist has recourse to colorimetric methods for determining several of these rarer elements. These procedures should be rather satisfactory for the purpose of comparison, which possibly expresses the requirement of the physiologist oftener than does absolute accuracy. There seems to be considerable applicability for colorimetric methods which may be accurate

to not over 95 per cent. It happens, however, that at least one such method is more sensitive than spectrographic procedure, excepting as the latter may be subject to refinement for evaluating the spectral lines. The difficulty of keeping up to date in this field is illustrated by current deficiencies in textbooks and manuals, as compared with the literature.

Boron

This element is subject to loss in ashing, primarily because of the formation of a volatile ester with glycerol from samples of high fat content. Preliminary impregnation of the tissue with a solution of either magnesium nitrate or sodium carbonate surmounts this difficulty.

Use has been made of the capacity of boric acid to esterify readily with methyl alcohol, for the purpose of isolation by distillation. Addition of mannitol to the free acid enhances the activity of the hydrogen ion so that it may be titrated. This type of procedure, as recommended by the A.O.A.C. for fertilizer samples, is inadequate for the low concentrations of boron encountered in plant tissues.

Preference has recently been indicated for a supposedly highly sensitive color reaction of boric acid with turmeric. This effect should be concentrated by capillarity at the top of a strip of indicator paper used wickwise (**29**, vol. 1, p. 526). The experience of an associate of a member of this committee with the turmeric method has been seriously disappointing, even with care as to factors controlling evaporation. Berger and Truog (**3**) have reported on the use of quinalizarin for the colorimetric determination of boron in soils and plants. This modification of a procedure developed by Smith (**28**) depends upon the color resulting from reaction with quinalizarin, and is reported to have the marked advantage of being applicable directly to digests of the ash.

From information now available, it appears advisable to separate the boron by esterification as directed in the Methods of the A.O.A.C. (p. 33) using, of course, boron-free glass. In addition, it will be necessary to use a photoelectric colorimeter in order to draw distinctions between amounts of boron in the vicinity of one part per million of the dry matter. For this purpose the use of a filter to transmit in the region of 6200 Å is advisable.

Attention may be called to a spectrometric procedure of Chalfee and McHargue (**7**). In this method the extinction coefficient of the spectrum of methyl borate is determined by means of a solution of potassium permanganate. The authors estimate that experienced machinists and glass technicians can assemble the apparatus at an expense of about $25.

Copper

The methods of the A.O.A.C. describe a colorimetric determination of this element in the form of its xanthate. A more sensitive procedure seems to be

available in the use of dithizone (phenylazothionoformic acid phenylhydrazide). This reagent reacts with a number of metals (see, for example, **18**). In presenting the dithizone method for copper, SNELL and SNELL (**29**, pp. 168–169) discuss some of its limitations. The requirements for preparation of reagents are somewhat exacting.

More reliable methods for copper involve the use of either pyridine thiocyanate (BIAZZO procedure, **4**) or diethyl dithiocarbamate (as used by CALLAN and HENDERSON, **6**). The former is the less complicated, while the latter is the more sensitive method. For application of the simpler procedure, see ELVEHJEM and HART (**11**), and for the other consult HADDOCK and EVERS (**15**).

IRON

The A.O.A.C. (**2**, p. 122) gives concise directions for the colorimetric determination of this element as ferric thiocyanate. SNELL and SNELL (**29**, pp. 283–298) discuss in detail the exacting requirement for avoidance of contamination, and the preparation of reagents. The latter recommend the addition of iron salt to the standard, and dilution as required, to duplicate the unknown. It is generally recognized that greatest reliability is secured by transferring both unknown and standard to a 1 : 1 mixture of amyl alcohol and ether for colorimetric comparison.

MANGANESE

It seems to be standard procedure to convert this metallic element to the form of potassium permanganate for determination. Apparently the procedure of the A.O.A.C. (**2**, p. 125) is fully acceptable.

ZINC

This element has been isolated commonly as the sulphide. SNELL and SNELL (**29**, p. 346) give directions for determining it nephelometrically (basis of turbidity) as the ferrocyanide.

When extreme accuracy is not essential, the dithizone (diphenylthiocarbazone) method of HIBBARD (**17**) may well be considered. This rapid procedure recovers 90 to 95 per cent. of zinc from amounts ranging from 0.001 to 0.100 mg. It entails scrupulous care for the use of zinc-free reagents and apparatus, to the extent of lubricating stopcocks by means of water. Interfering metals are eliminated by the application of solubility relations so that 3 to 30 gamma (thousandths mg.) of zinc are determined titrimetrically.

When extreme accuracy is desired, it will be well to consider the method of TODD and ELVEHJEM (**30**). Although their procedure seems to be involved, it is supposed to simplify and expedite the determination of this metal. Copper is separated from zinc by manipulation of the sulphides, and the latter metal is converted to its double phosphate with ammonium. A path is thus provided for determining zinc through its phosphorus equivalent.

The inorganic fraction of mineral elements

In numerous cases the significance of mineral determinations could be strengthened substantially if the relatively large proportion of the given element which existed in unassimilated form could be determined directly. Some time ago analysts gave attention to this issue. Perhaps phosphorus has been considered more frequently than other elements in this respect. Notable examples in this field are the papers of FORBES, BEEGLE, and WUSSOW (**12**), and CHAPIN and POWICK (**8**). As in other attempts to deal with the inorganic state, the problem appears to be that of inducing normal precipitation by overcoming the protective effects of colloids (especially of proteins). At the same time one must avoid the use of either reagents or conditional factors which might induce hydrolytic cleavage of phosphoric acid from organic compounds. FORBES and associates were unsuccessful in attempting to use either dilute watery or alcoholic solutions of mineral acids for extracting the inorganic phosphorus. On the other hand, CHAPIN and POWICK seem to have attained notable success in overcoming interference from proteins by extracting with dilute picric acid.

In recent years interest has developed in the determination of iron in the inorganic form. KOHLER, ELVEHJEM, and HART (**20**) have reported on the development of a procedure for determining available iron in foodstuffs by colorimetric use of the reaction with α-α'-bipyridine. They have encountered particular difficulty from the presence of tissue pigments. With fresh samples, interference from this source has been largely overcome by precipitation with lead acetate; but the difficulty has persisted with dried samples. An assistant to a member of this commitee was unable to apply the procedure satisfactorily to fresh potato tuber tissue.

Despite the paucity of methods, and the unsatisfactory nature of experience in this field, there appear to be elements of hope in the situation. If readers of these recommendations either have experience of value, or find it possible to extend the study of determining inorganic elements, it is hoped that they will make the experience available by publication. In this connection it may be well to consider the possible use of dialysis, and especially of electrodialysis under controlled voltages, as means of determining the degree of freedom of mineral elements. Examples of the application of these procedures are to be found in the literature.

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ABSORPTION AND MOVEMENT OF RADIOPHOSPHORUS IN BEAN SEEDLINGS

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Experimentation

Preliminary results using the radioactive isotope of phosphorus (P^{32}) as a tracer atom in Red Mexican beans (**2**) demonstrate the value of the method and its general application to plant physiological problems. Wherever it is desirable to follow the path or metabolism of single atoms, this procedure will prove extremely valuable.

The equipment used for the detection and measurement of the radiophosphorus consisted of a NEHER-HARPER high speed Geiger counter circuit (**14**) in connection with a direct-reading counting rate meter for random pulses as designed by GINGRICH, EVANS, and EDGERTON (**5**). A Geiger counter tube with a very thin glass window (**4**) proved satisfactory. The samples to be measured were held in cellophane cones which in turn were suspended in the concave sensitive area of the counter tube. Only a relatively small percentage of the total beta radiation emitted by the radiophosphorus entered the cathode cylinder of the counter tube; however, the geometric conditions remained constant for all determinations, and the counts per minute recorded represent a constant percentage of the total emanation. Results represent comparative amounts rather than absolute quantities.

The radiophosphorus was obtained through the courtesy of Dr. E. O. and Dr. E. H. LAWRENCE, of the Division of Radiation, University of California. It was made by bombarding 8-million volt deuterons against red phosphorus, which in turn was converted into sodium phosphate. The radiophosphorus-containing nutrient solution was made as follows:

Na_2HPO_4	0.0075 M	(contains the radiophosphorus)[1]
KCl	0.0020 M	
$Ca(NO_3)_2$	0.0020 M	
$MgSO_4$	0.0010 M	

The solution received continuous aeration. The conditions were not favorable for very rapid transpiration, as the light intensity fell from 2000 to 200 foot candles during the experiment. The temperature varied between 21° and 24° C., but was for the greater part of the experiment between 22° and 23° C.

Bean seeds, variety Red Mexican, were germinated and the plants grown in a complete nutrient solution until the fifth alternate leaf was unfolding.

[1] The final nutrient solution contained 0.76 μc/cc. of radiophosphorus.

They were then sorted for uniformity and three selected for tracer work. The first plant was placed in the radiophosphorus-containing nutrient solution for one hour, the second for two hours, and the third for four hours. After the required time, each was removed, the roots washed carefully and the plant dissected, dried in a 75° C. oven, ground to a fine powder and 10 mg. samples weighed for analysis. The remainder of the root tissues of each plant was extracted with ether in a Soxhlet, and then dried and extracted for three hours with successive portions of hot distilled water. The ether extract was evaporated, and determinations of radiophosphorus made on the residue. Aliquots of the water extract were also assayed. The results are shown in table I.

TABLE I

DISTRIBUTION OF RADIOPHOSPHORUS IN BEAN PLANTS GROWN IN NUTRIENT SOLUTION CONTAINING 0.76 μC/CC. RADIOPHOSPHORUS
(EXPRESSED IN COUNTS / MINUTE / MG. OF FRESH TISSUE)

PLANT ORGANS	TIME IN NUTRIENT SOLUTION												
	1 HOUR			2 HOURS			4 HOURS			COMBINED ROOTS			
	TISSUE	ETHER	WATER	TISSUE	ETHER	WATER	TISSUE	ETHER	WATER	WATER	ETHER	2% HCl	INSOL.
Roots	8.3	0	2.7	25.3	0.07	5.7	33.5	0.15	10.5	29%	1%	61%	9%
Hypocotyl and stem	0.0			1.1			0.6						
Opposite leaves	0.0			0.0			0.9						
1st alternate leaflets	0.0			0.0			0.5						
2nd " "	0.0			0.0			0.8						
3d " "	0.0			0.0			0.8						
4th " "	0.0			0.0			1.2						
5th " "	0.0			0.0			1.3						
Nutrient solution			11.8			11.0			10.4				

After finding that the water extraction removed only a comparatively small percentage of the total radiophosphorus, it was considered desirable to investigate the remainder. The tissue residues from the three roots were combined and extracted with 2 per cent. HCl. The phytin fraction is normally removed by this procedure and determined quantitatively by titration (**1**). The presence of ferric iron in the tissues, however, caused the precipitation of the insoluble iron-phytic acid complex, which remained in the tissue residue and consequently was not determined as such. The distribution of radiophosphorus in the combined roots can be calculated, and is as follows:

Water soluble	29%	2% HCl	61% (not phytin)
Ether soluble	1%	Insoluble	9%

The water soluble radiophosphorus in the tissues approaches that in the nutrient solution at the end of a 4-hour period.

Discussion

These results are of interest because the method employed enables the experimenter to follow absorption, movement, and accumulation of a nutrient salt in an intact plant.

Table I shows that the uptake of phosphorus under the conditions of the experiment was quite rapid. Radiophosphorus could be detected in the topmost leaves of the plant, a distance of approximately one meter from the base of the hypocotyl, within four hours after the roots were placed in the radiophosphorus-containing nutrient solution. This rate of uptake and movement is not as rapid as that reported by CRAFTS and BROYER (**3**) who detected Br in the xylem exudate of squash within 30 minutes after exposure of the roots to a solution containing 800 p.p.m. of KBr. With the bean plant, radiophosphorus was detected in the basal part of the stem within two hours after administration of radiophosphorus. It is very interesting to note the large amount of total radiophosphorus which was associated with the roots of the bean plants before appreciable quantities moved upward in the stem. It is not advisable to consider the total amount as being absorbed phosphorus, as relatively much may have been merely adsorbed on the surfaces. That which may be removed by hot water may represent more nearly the fraction which is normally free to move into the xylem vessels, and it can be seen from table I that the water soluble radiophosphorus associated with the roots approaches that in the nutrient solution after four hours of absorption. The relationship of the various fractions studied to the concentration of radiophosphorus in the nutrient solution can be seen from table I. It can also be seen that the time required for the movement of radiophosphorus into the stem corresponded fairly well with the time at which the total radiophosphorus associated with the roots reaches the same concentration as that in the nutrient solution. The proper interpretation of this point is questionable because of limited data.

The path of movement of radiophosphorus in the stem is unquestionably the xylem (**15**). It is impossible to account for the distribution throughout the plant assuming any other path. If movement were through the phloem (**6**) the concentration gradient in the aerial parts would necessarily be the opposite to that actually found. It appears, from the small amount of radiophosphorus in the hypocotyl (**2**) that on arrival of the radiophosphorus in the xylem tissue it is "swept" into the aerial parts by the transpiration stream. The distribution of radiophosphorus in the aerial parts corresponds very favorably to the transpiration rates of various leaves (**13**).

HEVESY, LINDERSTRØM-LANG and OLSEN (**7, 8**) present data from which

they contend that phosphorus migrates from leaf to leaf within the plant. They grew sunflower plants in a complete nutrient solution until a first set of leaves was formed, then transferred them to a nutrient solution containing radiophosphorus while a second set of leaves was formed. They then reason as follows:

". . . then we must distinguish between two extreme cases: (a) the phosphorus atoms do not migrate; (b) the phosphorus atoms migrate. In case (a) labeled phosphorus atoms should only be found in the upper leaves; in case (b) the labeled phosphorus atoms should be equally distributed between the upper and lower leaves . . ." (**8**).

Analysis showed that radiophosphorus moved into the lower leaves as well as the upper ones. This is, of course, no evidence for exchange of phosphorus atoms from leaf to leaf as the authors contend. It is merely evidence that phosphorus moved directly into the lower leaves as well as the upper ones through the vascular tissues supplying those leaves, and not that the radiophosphorus moved into the upper leaves only, and was then exchanged to the lower leaves. The same criticism applies to their work on maize (**8**). The above experiments were not properly designed to show migration of phosphorus from leaf to leaf. It is possible, however, for phosphorus to migrate from one leaf to another (**11**) chiefly upward, but it should be emphasized that the data of HEVESY *et al.* are not concerned with this type of exchange. A most interesting relationship between the work of MACGILLIVRAY (**11**), on the re-utilization of phosphorus by the tomato plant as the supply to the roots is removed, and the work of MASON and MASKELL (**12**) on the movement of phosphorus in the cotton plant, can be shown. The latter authors demonstrate that after the delivery of phosphorus to the leaves, via the xylem, there may be a downward movement through the phloem. They state that the downward movement in the phloem may be in excess of the amount required by the roots, and some of the mobile phosphorus may find its way back into the xylem and reascend the stem. This is the type of evidence which should be used to demonstrate migration of phosphorus atoms from leaf to leaf, and it is the only sense in which the term "migration" has meaning. No attempt seems to have been made to correct the erroneous impression of HEVESY *et al.*, as the inference is repeated in the new edition of "A Manual of Radioactivity" (**9**). It is hoped that, by calling attention to the above interpretation, a more correct appraisal of radiobiological activity may be made.

In tracing the movement of radiophosphorus in the bean plant, a concentration gradient has been found which corresponds to a transpiration differential. Accordingly, the transpiration stream delivers phosphorus to the leaves, where it accumulates as water is evaporated. This steepens the gradient in the phloem and allows a downward movement through that

tissue. If the supply of phosphorus to the root is suddenly removed, a diffusion of mobile phosphorus toward the xylem vessels would take place, and upon entry into the transpiration stream the ions would be "swept" upward again. This system of forces and gradients would account for the movement of ions which are water soluble, and which are known to accumulate in the upper parts of the plant as the supply to the roots is reduced or removed.

The very small amount of radiophosphorus in the ether soluble fraction within four hours after exposure to the nutrient solution is interesting, but previous work by WEBSTER and DALBOM (**16**) has shown that lipoid phosphorus constitutes only a small fraction of the total phosphorus of the mung bean. HEVESY and PANETH (**9**) state that radiophosphorus can enter into the phosphatide molecule only during its synthesis (in animals), and no evidence for the exchange of a newly acquired phosphorus atom for one already in chemical combination in a phosphatide molecule could be found. In the experimental work with the bean plant, it is highly improbable that ample time for much phospholipin synthesis was allowed, as the concentration of ether soluble radiophosphorus is still increasing at the end of four hours, the maximum period of contact with the nutrient solution.

Summary

1. The movement of radiophosphorus was traced throughout the bean plant. The total phosphorus associated with the roots was twice as great as the concentration in the nutrient solution before appreciable quantities entered the aerial parts.

2. The amount of water soluble phosphorus in the roots at the end of a four-hour period was equal to that in the nutrient solution.

3. Movement was rapid, and followed the transpiration stream.

4. Accumulation was greatest in the uppermost leaves.

5. An explanation of movement and accumulation is offered.

6. Criticism of the interpretations of HEVESY, LINDERSTRØM-LANG and OLSEN is offered.

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RESPONSES OF VARIOUS SPRING WHEATS TO VERNALIZATION

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(WITH TWO FIGURES)

Introduction

There is little information in the literature regarding North American varieties of spring wheat which are responsive to vernalization treatment. The same variety has been reported by different investigators to react positively or negatively to treatment. For example, VAN HOEK (**3**) obtained acceleration of maturity when spring wheat was vernalized, but treatment of the same variety by GMELIN (**1**) gave no acceleration of flowering. WORT (**4**), using the same treatment with two different samples of Marquis wheat, found that the acceleration of flowering was 21 days in one case, and 2.6 days in the other, although growing conditions were similar. KOSTJUCENKO and ZARUBAILO (**2**) and others suggest from experiments with cereals grown in northern latitudes that at least part of the stage of vernalization (thermostage) may be completed during ripening and that this partial completion may reduce subsequent reaction to treatment. The environment, acting on a developing seed, affects the behavior of the plant produced by the seed.

Samples of various spring wheats (table I) obtained from the Departments of Agronomy at the State Universities of Illinois, North Dakota, and South Dakota,[1] were vernalized in an attempt to determine which varieties respond to vernalization and if varieties grown in different areas respond differently.

Methods

The procedure used for vernalization was as follows: (1) the amount of water, given in the formula used, was added to 20 gm. of seed in Petri dishes; (2) the seeds were allowed to germinate for 24 hours at 20° C.; (3) the Petri dishes were then stored for the required lengths of time in mechanical refrigerators at the temperatures given in the formulas. The lids of the Petri dishes were removed daily and the seeds aerated for a few seconds.

The factors in a formula in order are length of chilling in days, temperature during chilling in degrees C., and amount of water added in percentage of the original air-dry weight of the seed. Three formulas were used: (1) for early varieties, 6:10:50; (2) for medium varieties, 9:6:50; and (3) for late varieties, 12:4:50. These formulas are referred to by number in table I. Control seed was germinated 24 hours at 20° C., the water added being 50 per cent.

[1] The writer is indebted to Dr. G. H. DUNGAN of the University of Illinois; Dr. S. P. SWENSON of the University of South Dakota; and Dr. L. R. WALDRON of the University of North Dakota for the supplies of seeds used.

Five plants of treated or control grain were grown in 6-inch pots filled with well-mixed loam soil. Three replicates were used. All plantings were made on Sept. 9, 1939. Daylight was supplemented by 200-watt mazda lamps suspended 3 feet above the pots to give a 16-hr. day for the first 10 days, and an 18-hr. day thereafter. The plants were watered once a day. Flowering was assumed to have occurred when the anthers were first extruded.

Results and discussion

The results are recorded in table I. The source of the grain is stated in

TABLE I

VERNALIZATION TREATMENTS, AND ACCELERATION OF FLOWERING OF SPRING WHEATS

NAME OF VARIETY	SOURCE	EARLINESS RATING BY SOURCE	VERNALIZATION FORMULA	AVERAGE TIME TO FLOWER		ACCELERATION OF FLOWERING
				VERNALIZED	CONTROL	
				days	*days*	*days*
Bluestem	S.D.†	L	3	49.5	47.8	*−1.7
Caesium	S.D.	ML	2	46.1	48.0	1.9
Ceres	S.D.	ME	2	46.3	47.3	1.0
Comet	Ill.¶		2	48.7	52.4	3.7
General San Martin	Ill.		2	38.9	39.3	0.4
Haynes Bluestem	Chicago	L	3	49.0	49.1	0.1
Hope	S.D.	M	2	51.5	48.4	−3.1
Illinois 1 (Station)	Ill.		2	43.0	44.0	1.0
Illinois 1 (Mann)	Ill.		2	44.5	44.0	−0.5
Komar	Ill.		2	47.5	49.9	2.4
Marquis	S.D.	L	2	46.5	49.9	3.4
Marquis	N.D.‡	ML	2	49.1	47.6	−1.5
Marquis	Chicago	ML	2	47.1	48.9	1.8
Mercury	S.D.	ME	2	42.0	42.5	0.5
Merit	S.D.	ML	2	47.8	48.8	1.0
Ns2822	N.D.	VE	1	43.4	43.2	−0.2
Pilot	S.D.	ME	3	44.3	43.8	−0.5
Pilot	N.D.	L	3	48.4	49.7	1.3
Power	N.D.	L	3	44.8	46.8	2.0
Premier	S.D.	E	2	43.8	44.7	0.9
Premier	N.D.	ME	2	42.4	43.1	0.7
Progress	Ill.		1	43.0	43.1	0.1
Purdue	Ill.		2	42.2	45.3	3.1
Quality	S.D.	VE	1	37.9	38.3	0.4
Reliance × Prelude	S.D.	M	2	52.5	53.5	1.0
Renown	Ill.		1	41.8	42.6	0.8
Renown	N.D.	E	1	43.3	43.5	0.2
Reward	S.D.	VE	1	39.2	40.2	1.0
Rival	S.D.	ME	2	40.5	43.5	3.0
Rival	N.D.	ME	2	48.0	49.0	1.0
Sturgeon	Ill.		2	43.6	45.4	1.8
Thatcher	S.D.	E	1	45.8	45.4	−0.4
Thatcher	Ill.		1	48.6	47.8	−0.8
Thatcher	N.D.	E	1	46.8	48.5	1.7
Triumph	S.D.	VE	1	39.5	40.2	0.7
Vesta	S.D.	ME	2	41.3	40.7	−0.6

* Minus sign indicates a retardation in flowering.
† S.D. = South Dakota.
¶ Ill. = Illinois.
‡ N.D. = North Dakota.

the second column and the rating of the wheat by local authorities is on the basis of very early (VE), early (E), medium early (ME), medium (M), medium late (ML), and late (L).

An examination of the table shows that a total of 27 samples responded to vernalization by acceleration of flowering and 9 were retarded by treatment. This ratio of 3 : 1 holds for the three main groups, early, medium, and late. Accelerations or retardations of less than one day, however, are probably insignificant. In some cases all samples of the same variety obtained from two or more sources (Marquis, Pilot, Premier, Renown, Rival, and Thatcher) responded similarly, and in other cases differently to treatment. Thus the accelerations of flowering of samples of Premier, obtained from North Dakota and South Dakota, were practically the same, being 0.7 and 0.9 days respectively. Marquis from South Dakota was accelerated in its flowering by 3.4 days; from North Dakota, retarded by 1.5 days; and a sample obtained in Chicago responded to treatment with an acceleration of 1.8 days. In a number of cases the relative flowering time of the samples grown in the greenhouse, did not correspond to the earliness rating given by the institutions supplying the grain.

The average height of the vernalized plants was slightly greater than

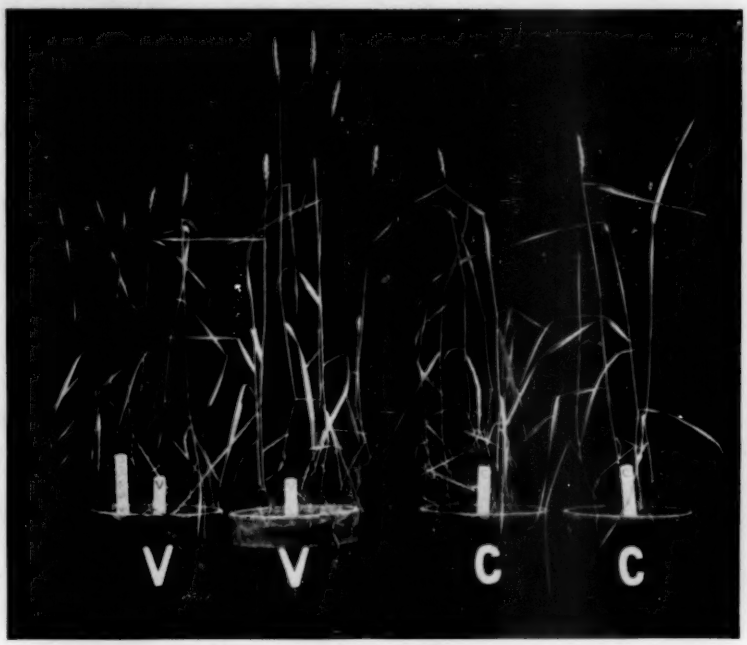

FIG. 1. Representative plants, South Dakota Marquis wheat, age 46 days. V = vernalized, C = control.

control in most cases; for example, Marquis (South Dakota) was 10.5 per cent. taller, and Thatcher (North Dakota) was 7.6 per cent. taller than their controls when measured on Oct. 31. The increased height was due to longer internodes. The appearance of these plants on Oct. 25 is shown in figures 1 and 2.

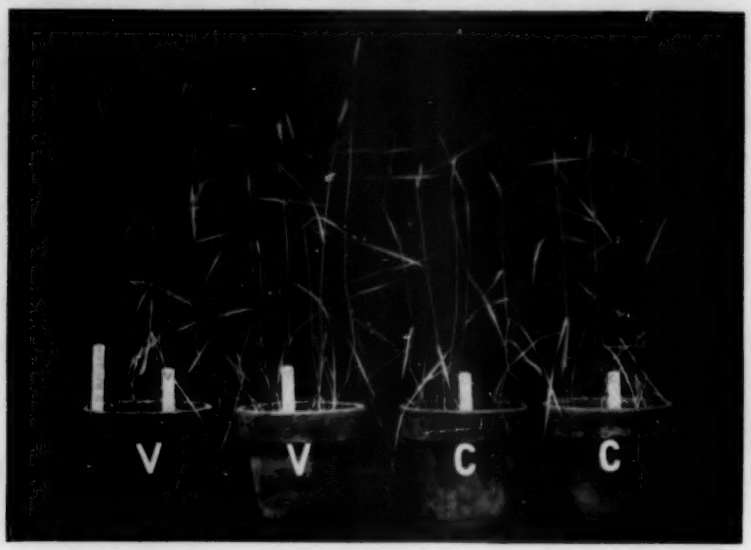

FIG. 2. Representative plants, North Dakota Thatcher wheat, age 46 days. V = vernalized, C = control.

The varied responses of the cereals make it very difficult to correlate the available weather data covering the period of flowering of the parent plant, and ripening of the seed later treated, with the seed's reaction to vernalization. It can be said, however, that the five varieties whose flowering was accelerated most by treatment—Comet (3.7 days), Marquis (3.4 days), Purdue (3.1 days), Rival (3.0 days), and Komar (2.3 days)—had been grown in South Dakota or Illinois where the temperature during flowering and ripening was higher than that in North Dakota. Of six varieties that had been grown in North Dakota and some other state only two of those produced in the more northerly area gave a greater response when tested.

While the vernalization formulas used may not have been optimum for all varieties, the results indicate those which are most responsive to treatment and hence most favorable for vernalization studies.

Summary

Thirty-six samples of various spring wheats from different regions in the United States were vernalized; of these, 27 responded with an acceleration,

and 9 with a retardation of flowering. Some varieties responded differently to treatment depending on the location where the seed had been produced, others responded similarly irrespective of their origin. The samples that responded most to vernalization were those that had been produced in areas with relatively high temperatures during the flowering and ripening of the parent plant.

DEPARTMENT OF BOTANY
THE UNIVERSITY OF CHICAGO

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BRIEF PAPERS

EFFECT OF CHLOROPICRIN IN THE SOIL SOLUTION ON PLANT GROWTH[1]

FRANK L. STARK, JR., FRANK L. HOWARD, AND JOHN B. SMITH

(WITH ONE FIGURE)

Previous experiments have shown that increased plant growth in chloropicrin-treated soil cannot be entirely explained on the basis of increased available nitrogen. Investigations have been carried on, therefore, to determine whether or not small traces of chloropicrin, which may remain in the soil following treatment, have any direct physiological stimulation upon plant growth. Several workers have concluded that volatile antiseptics used for soil treatment, when present in small quantities, have a stimulating effect on plant growth. KOCH (3) working with carbon disulphide and ether, concluded that residual traces of these substances affect the roots of plants, and he advanced the theory of direct plant stimulation. FRED (1) working with carbon disulphide and ether, holds that the increased growth of plants following the use of antiseptics in the soil depends essentially upon the stimulation of the plant itself. He finds (2), also, that in sand cultures supplied with soluble plant food, carbon disulphide favors the growth of certain plants. More recently, OLDENBUSCH (4) also concludes that small quantities of antiseptics exert a stimulating effect upon plant growth.

Because of the evidence cited the following experiment was set up to determine if it is possible that the beneficial effects of treating the soil with chloropicrin are of a direct stimulative nature. Ten drip culture pots were filled with washed, screened sand. Twenty-one buckwheat seeds were planted in each pot and grown by a drip culture method.

The culture solution contained the following concentrations of salts: calcium nitrate, 0.005 M; potassium dihydrogen phosphate, 0.001 M; potassium nitrate, 0.005 M; magnesium sulphate, 0.002 M; manganese sulphate, 0.00001 M; and boric acid, 0.0001 M. Dilute concentrations of chloropicrin were provided by adding varying amounts of a stock solution of chloropicrin to 180 liter volumes of culture solution. The flow of the solution was adjusted to a rate of about 6 drops per minute, approximately a liter per day.

The buckwheat plants were grown for thirty-five days and were then harvested. Because of the comparatively small root system developed by the buckwheat, the plants were cut off at the base of the stem and only the tops were weighed. The results obtained are shown in table I.

[1] Published by permission of the Director of Research as Contribution no. 557 of the Rhode Island Agricultural Experiment Station.

TABLE I

EFFECT OF VARIOUS CONCENTRATIONS OF CHLOROPICRIN ON THE GROWTH OF BUCKWHEAT

POT	DILUTION OF CCl_3NO_2	NUMBER OF PLANTS	AVERAGE WEIGHT PER PLANT	WEIGHTED AVERAGE OF DUPLICATE TREATMENTS (GM. PER PLANT)
			gm.	*gm.*
1	No chloropicrin	20	5.78	5.89
2	1/500,000	16	4.00	4.10
3	1/1,000,000	20	4.45	4.47
4	1/2,000,000	20	5.45	5.09
5	1/3,000,000	20	5.38	5.11
6	No chloropicrin	18	6.00	
7	1/500,000	15	4.20	
8	1/1,000,000	19	4.50	
9	1/2,000,000	20	4.73	
10	1/3,000,000	20	4.85	

The plants came into flower at the same time regardless of treatment. All plants except those subjected to the highest concentration of chloropicrin were approximately the same height (fig. 1). The green weight was in-

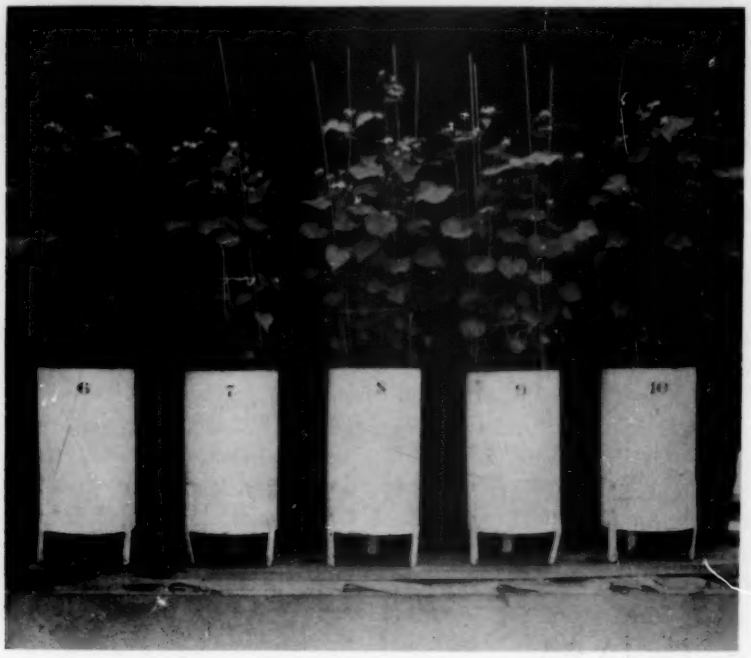

FIG. 1. Buckwheat plants grown by means of drip culture solution to which had been added various amounts of chloropicrin. Pots nos. 7 through 10 received decreasing amounts of chloropicrin, and pot 6 received no chloropicrin.

versely dependent upon the concentration of chloropicrin. In no case where chloropicrin was present, even in the most minute quantities, were the plants heavier than those developed in the plain culture solution. It is possible that concentrations lower than those used might stimulate plant growth. This is very improbable, however, because the lowest concentration was only one part of chloropicrin in three million of the culture solution.

In conclusion, the data appear to indicate that traces of chloropicrin in the soil solution are detrimental rather than stimulative to plant growth.

THE RHODE ISLAND AGRICULTURAL EXPERIMENT STATION
KINGSTON, RHODE ISLAND

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EFFECT OF NITRATE OF SODA ON THE RESPONSE OF SPINACH TO LENGTH OF DAY[1]

JAMES E. KNOTT

(WITH ONE FIGURE)

It is the common observation of growers that spinach plants which are closely crowded in the row, especially on mineral soil, will produce seedstalks more rapidly than where each plant has more room for development. On the peat soils of New York, spinach plants of the "slow bolting" varieties develop seedstalks very slowly under the long photoperiod of the summer months. It has been shown by WILSON and TOWNSEND (**2**) that the content of nitrate nitrogen in these soils may exceed 500 p.p.m. at a depth of 4 to 8 inches. This is equivalent to about 6.5 tons of nitrate of soda per acre foot. Considerably higher values are shown for the upper 4 inches of soil.

SCHAPPELLE (**1**), in studying the reproductive growth of Marchantia, radish, and spinach, found that cultures given distilled water without the addition of any nutrients were the first to respond to a photoperiod of 14 or 15 hours.

Iron drums 22 inches in diameter and 17 inches in depth were used as soil containers in this experiment. They were provided with a drainage hole in the bottom. Six drums were filled with a Dunkirk sandy loam soil of medium fertility and placed outside of the greenhouses where they were exposed to the prevailing weather and light conditions of the spring months at Ithaca. Seed of the Long Standing Bloomsdale variety of spinach was planted April 26, 1934. On May 7, the seedlings were thinned to a stand of 20 uniformly-sized plants in each drum. Nitrate of soda was applied to three of the drums beginning May 14. The rates of application were as follows: one ounce to each drum on May 14 and 21, June 11 and 18; four ounces to each drum on May 28 and June 5. The drums were watered well each time the nitrate was applied and an equal quantity of water was put on the check drums. The total application of nitrate of soda to each drum was equal to about 6 tons to the acre. The quantity of water applied, however, was such that it is likely that much of the nitrate was washed down to the lower levels of soil especially around the sides of the drums.

The spinach plants receiving nitrate were stockier, with foliage that was thicker, a darker green in color, and somewhat more savoyed than those which received no nitrate. Elongation of the central axis of some of the plants was first evident in both the treated and untreated plants on June 5. This was 40 days after planting. The photoperiod was then 15.1 hours. Al-

[1] Paper no. 192. Department of Vegetable Crops at Cornell University, Ithaca, New York.

though elongation commenced in some of the plants in all six drums at about the same time, most of the nitrated plants were slower in beginning seedstalk elongation than were those not nitrated. This is shown in table I by the percentage of seedstalks on June 14.

The heights of the seedstalks were measured on June 22. The means with their probable errors are given in table I. None of the tallest plants in the

TABLE I

PERCENTAGE OF PLANTS SHOWING ELONGATION OF THE CENTRAL AXIS ON JUNE 14, AND THE AVERAGE HEIGHT OF SEEDSTALKS ON JUNE 22 OF LONG STANDING BLOOMSDALE SPINACH GROWN WITH AND WITHOUT SIDE-DRESSINGS OF NITRATE OF SODA

No nitrate added			Nitrated		
Drum	Plants showing seedstalks on June 14	Average seedstalk height on June 22	Drum	Plants showing seedstalks on June 14	Average seedstalk height on June 22
	%	*mm.*		%	*mm.*
A	80	241.8 ± 22.2	B	52	110.6 ± 17.7
C	77	230.3 ± 21.9	D	68	111.0 ± 16.2
E	88	157.1 ± 16.2	F	59	103.1 ± 14.2
Average of all plants		209.3 ± 12.4	Average of all plants		108.3 ± 8.9

FIG. 1. Foliage characteristics and relative seedstalk height of spinach plants photographed June 19. The plants in drum A received no nitrate while those in drum B were heavily nitrated.

nitrated drums was as tall as those not nitrated. This indicates that the seedstalks in the plants receiving nitrate elongated more slowly than did those to which no nitrate was applied. The type of growth on June 19 in drums A and B is shown in figure 1.

The large quantity of nitrate applied to this sandy loam soil has delayed seedstalk initiation and development. These results emphasize the importance of a consideration of the level of fertility of the soil in which are growing plants for use in photoperiodic experiments.

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QUANTITATIVE STUDY OF THE PRODUCTION OF ETHYLENE BY RIPENING MCINTOSH APPLES

R. C. NELSON

(WITH ONE FIGURE)

Introduction

In an earlier paper, (**4**), the author has described the relationship which exists between ethylene production and keeping quality in several varieties of apples, and noted the similarity in shape of the curves for respiration and ethylene production. In the present paper ethylene production has been studied in relation to respiration and the ripening process.

Material and methods

The method used was similar to that which had already been used by the author (**5**) in studies of the banana. The fruit was placed on a wire stand beneath a bell-jar together with a known amount of base to absorb the carbon dioxide formed in respiration. The carbon dioxide absorbed was replaced by oxygen under low constant pressure from a gasometer, thus maintaining a normal atmosphere in the jar while permitting the accumulation of emanations from the fruit.

After the fruit had been under the bell-jar about 20 hours, a sample of the gas in the jar was taken for analysis. The fruit was then aired in preparation for the next day's run. The amount of carbon dioxide produced was determined by diluting the base to a suitable volume, adding barium chloride to an aliquot to precipitate carbonate, and titrating the free base to phenolphthalein with standard hydrochloric acid. All results were reduced to a 24-hour basis and are so expressed.

In the present experiment McIntosh apples obtained locally were used, 4.30 kg. of fruit being placed under the bell-jar. The experiment was carried out in a room maintained at 20° C.

Results

The results obtained are shown graphically in figure 1. At the beginning of the experiment the apples, judged by taste, respiratory activity, and the presence of starch, were unripe. The digestion of starch began on the fourth day, when the respiration was at its maximum (the climacteric) and was practically complete on the seventh day. The progress of starch digestion was observed by use of the iodine test on sections of tissue. The characteristic odor of ripe McIntosh apples was first present on the fifth day.

Respiratory activity increased rapidly up to the fourth day, after which it declined slowly. KIDD and WEST (**3**) have shown that this respiratory maximum at the time of ripening is characteristic of apples.

The rate of emanation of ethylene remained low during the first 3 days and then increased rapidly to the ninth day, after which it began to decline. The maximum of ethylene production occurred five days after the respiratory maximum.

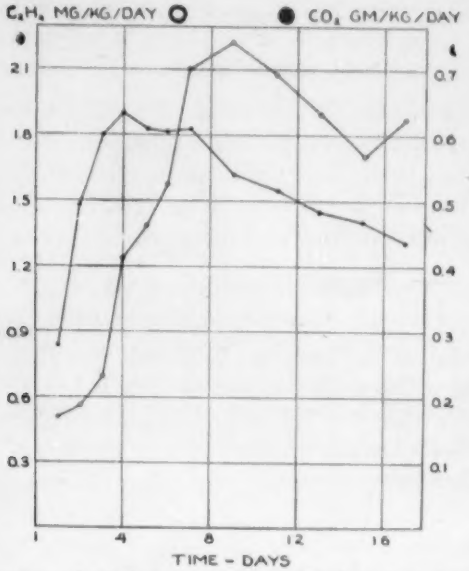

FIG. 1. Respiration and ethylene production of McIntosh apples during ripening.

Discussion

It will be observed that there is an inflection in the curve (fig. 1) of ethylene emanation on the fifth and sixth days corresponding with the period of active ripening. This inflection is probably attributable to the consumption of ethylene in the ripening process, such as the author has demonstrated in bananas (5).

The effects of ethylene on plant respiration are well known. DENNY (1) found that ethylene in concentration of 100 p.p.m. in air stimulated the respiration of lemons. HARVEY (2) has found that ethylene in concentration of 2 p.p.m. at first stimulated and later inhibited respiration of pea seedlings. One would predict, accordingly, that some relation would exist between ethylene production and respiration in the apple. No simple relationship appears in the present data. It is possible, however, that the low preclimacteric concentrations of ethylene are stimulatory, causing the onset of the climacteric, and that the higher concentrations are depressive causing the decline in respiration after the climacteric.

It seems likely that ethylene does play some essential part in the ripening process in both apple and banana, but it is not yet possible to say exactly what its rôle can be.

Conclusions

The rate of ethylene production in the McIntosh apple begins to increase rapidly after the onset of the climacteric, and reaches a maximum several days later than the respiratory maximum. Evidence is presented to show that, as in the banana, ethylene is consumed during the ripening process in McIntosh apple.

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FLAX VARIETIES COMPARED

J. C. Ireland

The introduction of a crop involves the question of quality as well as the environmental conditions under which profitable production may be undertaken. Varieties enter into the quality of the crop. In order to solve this problem in the case of flax, a comparative study of the available samples of seed was made, following the routine methods for "oil seeds." Samples were obtained from four states besides Oklahoma where flax is grown for the seed. Experienced seedsmen or experiment station workers labeled the varieties, and we assume that they are true to name. The object of our work has been to determine which variety is most desirable from the standpoint of oil and protein production and to determine if there is a climatic limitation for flax seed production.

The Wintons (1) quote Haselhoff, showing that flax obtained from widely separated areas has a fairly uniform composition. The differences might have been due to varieties or soil conditions.

Methods

The Official Methods of the A.O.A.C. were used in determining the mois-

TABLE I

Comparison of flax varieties from five states

Variety	Water	Protein	Oil	Iodine number	Ash	Weight 1000 seeds
	%	%	%		%	*gm.*
Kansas						
Bison	10.43	38.10	34.49	194.6	3.96	6.13
Linota	9.57	31.19	27.78	190.6	4.15	3.94
Redwing	9.02	33.87	24.02	199.3	3.14	4.22
Viking	8.67	35.37	33.82	200.0	3.00	6.18
Minnesota						
Bison	7.24	40.81	34.44	201.5	3.27	5.85
Redwing	10.15	29.44	24.96	177.4	3.08	4.28
North Dakota						
Bison	9.43	41.50	32.66	181.8	4.05	5.70
Buda	9.52	42.62	34.33	186.6	3.29	4.27
Linota	9.10	37.12	27.71	194.0	3.26	3.78
Oklahoma						
Redwing	8.71	31.56	25.89	170.3	3.53	4.29
Linota	9.13	33.54	28.20	179.4	2.82	3.81
South Dakota						
Bison	9.75	40.09	31.07	179.3	3.91	4.20
Buda	10.10	40.25	34.05	188.9	3.58	4.31
Linota	9.50	37.88	31.87	200.7	3.39	3.95
Redwing	8.78	36.50	29.39	195.8	3.53	4.72

TABLE II

SUMMARY OF FLAX VARIETIES

VARIETY	WATER	PROTEIN	OIL	IODINE NUMBER	ASH	WEIGHT 1000 SEEDS
	%	%	%		%	*gm.*
Bison	9.21	40.12	33.16	189.3	3.79	5.37
Buda	9.81	41.43	34.19	187.7	3.43	4.14
Linota	9.32	34.93	28.89	191.2	3.41	3.87
Redwing	9.16	32.84	26.06	185.7	3.32	4.38
Viking	8.67	35.37	33.82	200.0	3.00	6.18

TABLE III

SUMMARY OF FLAX SEED BY STATES

STATE	WATER	PROTEIN	OIL	IODINE NUMBER	ASH	WEIGHT 1000 SEEDS
	%	%	%		%	*gm.*
Kansas	9.18	34.63	30.03	196.1	3.56	5.11
Minnesota	8.69	35.14	29.70	189.4	3.17	5.02
North Dakota	9.35	40.31	31.56	187.8	3.53	4.58
Oklahoma	8.91	32.55	27.04	174.4	3.17	4.05
South Dakota	9.53	38.68	31.59	191.2	3.60	4.24

ture and protein content. For this determination, the seed was ground with a food chopper. The oil was extracted in Soxhlet extractors by petrol ether. The residue in the thimbles was used for the protein determinations. The Hanus iodine number was determined according to the Official Methods. The weights of 1,000 seeds are importont in showing the principal varietal difference in flax.

Results

Table I summarizes the data. Unfortunately, it was difficult to obtain samples of all varieties from all the states. Inspecting the results for the variety Bison, it may be observed that it is comparatively high in everything, wherever grown. Seed catologues describe it as a product of the North Dakota Experiment Station, resistant to rust and wilt. Buda is perhaps equal to Bison, but it seems to be available only in the Dakotas. The more widely distributed Linota and Redwing do not have the oil and protein values of the others. The Viking flax from Kansas is a golden-seeded variety that may become a very popular type.

Table II averages all the data from table I under the respective varieties. State summaries are included in table III. The apparent superiority of the seed from the Dakotas may be attributed to the fact that it was especially selected while those from other states were more nearly "field runs." Flax

variations appear to follow varietal differences, rather than the environmental factors found in different states.

Bison and Buda seem to be somewhat superior to other varieties, not only in composition but probably in disease resistance.

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NOTES

Annual Meeting.—The sixteenth annual meeting of the American Society of Plant Physiologists was held at Columbus, Ohio, December 28–30, 1939. The meeting was well arranged, and excellent programs were presented. For the efficient and painstaking service of the program committee, special notice and thanks are due to Dr. B. S. MEYER of Ohio State University, chairman of the committee, and to his assistants, Dr. J. D. SAYRE and Dr. R. O. FREELAND. Everything that could be done to insure a comfortable and profitable meeting was done with dispatch. The officers, also, President JOHN W. SHIVE, and the Secretary, Dr. W. E. LOOMIS, gave freely of their time and energy to insure a successful meeting.

The meetings were featured by several interesting and valuable symposia. One of these, on December 29, participated in by horticulturists and plant physiologists, dealt with the *Physiological Processes of Plants in Relation to Temperature.* The papers were well prepared, and presented a remarkable array of facts regarding the influence of temperature upon the absorption of water and solutes, photosynthesis, growth, flowering, and fruiting behavior of plants. A very long symposium on *Photosynthesis* was held under the auspices of Section C, A.A.A.S. The papers presented in this symposium should be collected and published, so that all workers might be able to read and study them more carefully. A symposium on the teaching of plant physiology occupied a late hour on Friday evening, December 29, under the leadership of Dr. O. F. CURTIS, of Cornell University. It attracted a deeply interested group whose problems with the teaching of plant physiology are many and varied. These teaching discussions are helpful, and deserve encouragement. Each one carries away some practical ideas of improvement of technique which must in some measure lift the general level of our teaching programs.

The annual dinner, held in the Junior Ball Room of the Neil House, Columbus, drew a large group together. Approximately 200 members and friends enjoyed the fellowship of the evening. Dr. WALTER F. LOEHWING, retiring president, read a very interesting paper entitled *The Effect of Mineral Nutrients on Flower Physiology,* and President SHIVE called upon the life membership committee, Dr. EARL S. JOHNSTON of the Smithsonian Institution, chairman, to report the selection of a recipient of the sixteenth award of the CHARLES REID BARNES life membership. The report was received with great interest and applause. The committee deserves the highest commendation for its excellent service. The report of the award is made in the following note.

Taking everything into consideration, the sixteenth annual meeting was

very well organized, very successful, and reflects the fine service of the officers and committees responsible for its success.

Life Membership.—The sixteenth award of the CHARLES REID BARNES life membership was made to Dr. W. J. V. OSTERHOUT, who, owing to an unfortunate illness, was unable to be present to receive the award. Dr. OSTERHOUT, who has been connected with the Rockefeller Institute for Medical Research for many years, is the author of many papers dealing with the problems of permeability, antagonism, and bioelectric potentials in living cells. These contributions have won him world-wide recognition, and he has given a life-time of service in the field of plant physiology. His was among the first one hundred names to be starred in *American Men of Science* in 1906. Born at Brooklyn, New York, August 2, 1871, he was educated at Brown University, where he won his A.B. degree in 1893, and A.M. in 1894. After a period at Bonn in 1895–1896, he returned to the United States, and received his Ph.D. from the University of California in 1899. He was instructor at Brown University for two years, 1893–1895, and rose through the ranks from instructor to associate professor at the University of California from 1896 to 1909. In this latter year he was called to Harvard, where he served for 16 years. In 1925 he became a member of the Rockefeller Institute for Medical Research. He was a joint-editor of the Journal of General Physiology, and a member of the Board of Trustees of the Marine Biological Laboratory, Woods Hole, Massachusetts. He served also for some years on the Board of Directors of the Rockefeller Institute. He has been the recipient of many honors, degrees from Harvard (1925), and Brown (1926); membership in the National Academy of Sciences; and honorary membership in numerous foreign societies.

In making its selection, the committee has made a very happy choice, one by which the Society is deeply honored. We are grateful that Dr. OSTERHOUT has been added to the roster of CHARLES REID BARNES life members.

Southeastern Section.—In accordance with provisions of the constitution, a petition duly signed by a large group of members residing in the southeastern states came before the American Society of Plant Physiologists at Columbus, asking that they be granted a charter for the establishment of a Southeastern Section of the Society. The petition set forth the territory to be included, as follows: North Carolina, South Carolina, Florida, Tennessee, Mississippi, Louisiana, Alabama, Georgia, and the West Indies. The temporary officers are Dr. G. M. ARMSTRONG, of Clemson College, chairman, and Dr. T. J. HARROLD, University of Georgia, secretary. The petition was favorably received by the executive committee, and on presentation before the business meeting of the Society, was given a unanimous vote of approval.

The first meeting of the new Southeastern Section will be held in the near future, and permanent organization will be effected. We hope that the development of this section will prove beneficial to the region it serves. This is the third regional section, and the fifth section to be established. We extend congratulations to the officers and members upon the successful outcome of their efforts. They will automatically obtain representation upon the executive committee of the Society, through the election of a permanent chairman, who then becomes a member of the executive group. We wish the new section every success with the organization.

Program Committee.—The program committee for the seventeenth annual meeting at Philadelphia in 1940 will be headed by Dr. WILLIAM SEIFRIZ of the University of Pennsylvania. The success of a meeting depends very much upon the convenience of arrangements, and it is a great advantage to have the chairman of the program committee on the grounds, and thoroughly familiar with all of the problems. Philadelphia was the scene of the third annual meeting of the American Society of Plant Physiologists. During the fourteen-year interim great progress has been made; it should be fully reflected in the program and the attendance. Cooperation of all members is desired in making this meeting our most important one to date.

Manuscripts.—The editor-in-chief desires to repeat the request that all manuscripts be sent originally to the office of the secretary of the editorial board, Dr. WALTER F. LOEHWING, Department of Botany, State University of Iowa, Iowa City, Iowa. Serious delays will be encountered by papers sent directly to Chicago.

The method of handling papers was slightly modified at the Columbus meeting, to preserve the benefits of having the papers examined by competent readers, but to allow expedition in reaching a decision on the papers submitted directly to Dr. LOEHWING.

In view of the critical financial situation of many of our subscribers, we are requesting authors to put their work into as concise form as possible, with few tables and cuts, and few mathematical formulae. These are the most expensive items in the printer's statements. Text is relatively inexpensive, but may be shortened by concise treatment of methods and previous literature.

Drawings should be made not larger than 8.5×11 inches, which require reduction to one-half or one-third to fit the pages of PLANT PHYSIOLOGY. We can no longer accept rolls of enormous-sized drawings. Authors submitting larger drawings than regular size will be requested to send other copies of the right size, or photographic reductions, before they are submitted to the readers.

Reprint Orders.—It is desirable to have all reprint orders filed in the editorial office at Chicago. When orders are sent directly to Lancaster, the editor-in-chief cannot give the orders the attention that they should receive. There is no objection to the making out of official orders to the Science Press Printing Co., but these orders should be filed with the editor so that they may be transmitted to the publishers with the page proofs. The green order blanks should represent the entire number of reprints desired by the author and his institution. If the shipment is to be divided between the author and the institution, directions should be given for the two shipments on the blanks designed for this purpose, at the lower end of the reprint order blank. Errors are difficult to avoid unless the author cooperates with the editor-in-chief by sending full and complete information on the order, and the official papers issued by his institution.

Endowment Funds.—A slight change was made in the endowment fund accounting at the sixteenth annual meeting. The life membership endowments are to be consolidated with the general endowment to simplify the book-keeping. There has been a healthy growth of the general endowment during the last year or two. Those who are able to do so may aid in the building of these funds by purchasing journals that are over five years old. Gifts are always welcome, and the finance committee will be very glad to receive funds for investment at any time. A general endowment of $25,000 would enable us to publish all papers promptly. It would also enable us to use an occasional colored plate when color is necessary to an understanding of the results. Thus far, all colored plates used in PLANT PHYSIOLOGY have been presented by institutions interested in the appearance of the papers with colored plates. At the close of the fiscal year, June 30, 1940, a statement showing the condition of the endowment funds will be published, in the hope that many may be encouraged to help increase them.

Robert Marshall.—On November 11, 1939, death came to Dr. ROBERT MARSHALL, Chief of the Division of Recreation and Lands of the U. S. Forest Service. He had not quite completed his 39th year. He was born in New York on January 2, 1901. His degrees were obtained at Syracuse (B.S., '24), Harvard (M.F., '25), and Johns Hopkins (Ph.D., '30). For several years after completion of his forestry studies at Harvard, he was a member of the staff of the Northern Rocky Mountain Forest Experiment Station (1925–1928). He was engaged in research for four years, until 1932, when he again was connected with the Forest Service. He spent one year as collaborator, and then was made director of forestry and grazing in the Bureau of Indian Affairs in the Department of the Interior, a position which he held for four years. In 1937 he became Chief of the Division of Recreation and

Lands, the position he held at the time of his death. During the period of his research, he was a member of an expedition to the arctic regions of Alaska for the study of tree growth and civilization.

Dr. MARSHALL was a life member of the American Society of Plant Physiologists, was also a member of many scientific organizations, and a member of the Explorers' Club. It is unfortunate, indeed, that one so well prepared for his work, one who had made such rapid progress in his chosen field, should so early be taken from us. He is deeply missed and mourned by his many colleagues and friends.

Quantitative Biological Spectroscopy.—During recent years great use has been made of spectroscopy in the quantitative aspects of biological investigation. This is especially true in the quantitative estimation of pigments, and in the identification and purification of such substances. Dr. ELMER S. MILLER, of the University of Minnesota, has published a very valuable monograph on the applications of spectroscopy to biological problems under the title *Quantitative Biological Spectroscopy*. The publishers are the Burgess Publishing Co., Minneapolis, Minnesota. There are 18 chapters, covering concisely, but still thoroughly, the necessary material. It begins with a short history of optics, and an introduction to molecular spectra, then takes up the instruments, such as thermopiles, photoelectric cells, amplifiers, and spectrophotometric instruments. These chapters are followed by the methods of spectrophotometric and photoelectric photometry, the basis of absorption spectra study, sources of error, and the application of the methods to biological chemistry. Some of the later chapters consider the absorption spectra of vitamines, cytochromes, hemoglobin derivatives, lipids, and the analysis of binary, ternary, and quaternary mixtures. The work closes with a bibliography of 163 titles, annotated. The reviewer believes that many workers will find this book a mine of valuable information; for, whether we work in the field or not, we need the information to understand the work being done by spectroscopists. The contributions in this field have been extremely valuable, so the general worker should understand the nature and validity of the results obtained.

The book is mimeoprinted, with many illustrations, and occupies 213 pages. The price, $3.50, is very reasonable for a work of this type. Orders may be sent to Burgess Publishing Co., 426 South Sixth St., Minneapolis, Minnesota.

Elements of Plant Physiology.—It is with a great deal of pleasure that we announce the appearance of *Die Elemente der Pflanzenphysiologie*, by Dr. P. BOYSEN JENSEN, Professor of Plant Physiology at the University of Kopenhagen. This work first appeared as *Plantefysiologi* in the Danish

language. It has now been translated into German by Dr. FRITZ MATTICK of the Botanical Museum, Berlin-Dahlem. The work is well organized. The introductory chapter distinguishes lifeless and living material, and considers the influence of external and internal factors upon living organisms, and the stimulus responses of living organisms. The second chapter deals with the gross structure, and anatomy of plants, the chemical constitution of plant substances, and the submicroscopic structures, with accompanying surface phenomena. This chapter closes with a consideration of the behavior of plants toward extreme conditions of temperature, light, drought, hydrogen-ion concentration, and poisons.

Then follow chapters on material intake, material outgo, and translocation of material through the plant body; energy release and energy transformations; assimilation; growth and development, and orientation movements of plants and their organs. Special attention is called to the treatment of growth and responses. These chapters should be familiar to all teachers of plant physiology, as they represent the views of one of the world's greatest students of hormones in relation to plant responses.

The author's style is direct, the translation has been skilfully accomplished, and readers will not experience difficulty in reading it, if they are moderately proficient with German. We commend it to all students of plant physiology as a text with which they should be familiar. It is well illustrated (162 text figures), and is provided with a good index. The publisher of this excellent work is Gustav Fischer, Jena, who quotes it at RM 24 per copy in paper binding.

Magic Gardens.—Lovers of old-fashioned gardens will find much pleasure in reading *Magic Gardens,* by ROSETTA E. CLARKSON. It is a Macmillan Co. publication, and a fine addition to their long list of garden books. This work deals with fragrant herbs and savory-seeded plants, used more in bygone ages than now. The book is like a breath of fragrance out of an Elizabethan garden, and the illusion is maintained by numerous illustrations taken from ancient herbals and old books on gardening. One can find in it inspiration to have one's own garden of old-fashioned herbs, of anise, basil, burnet, caraway, sweet marjoram, rosemary, and thyme. Moreover, it has the value of an historical document, with its illustrations from such works as PARKINSON'S *Paradisus in Sole* (1629), EVELYN'S *French Gardiner* (1675), GERARDE'S *Herball* (1597), LIGER'S *Le Jardinier Fleuriste* (1763), LAWSON'S *A New Orchard and Garden* (1618), and many other early sources of information. It is recommended especially to those who practice gardening for the love of it, to those in whose memories linger the odors of freshly turned soil, of spring flowers, of luxuriant woods vegetation, of grandmother's sage and hoarhound. The cost of this book is only $3.00, and its possessors will receive large dividends in pleasure.

FRANK MARION ANDREWS
JULY 27, 1940

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